Loughborough University

Water Engineering Department

School of Civil & Building Engineering

Ph.D. Thesis

Academic Year 2013 – 2014

Patrick VAN LIERDE

Nucleation, Milk and Membranes as Modifications to Enhance Biological Phosphorus Removal in Activated Sludge.

Supervisor: Pr. Andrew D. WHEATLEY

March 2014

This Thesis is submitted in partial fulfilment of the requirements for the degree of Ph.D.

Abstract

Enhanced biological phosphorus removal (EBPR) was researched from the performance of a modified University of Cape Town (UCT), anaerobic-anoxic/nitrifying-aerobic process. The work focussed on high P influent where milk was compared to carbohydrates as exogenous added carbon and typical settled sewage. The results confirmed that at equal COD load in the influent (minimum COD:P (250:5) ratio for EBPR), milk always provided sufficient soluble substrate than the carbohydrate mix, but also improved the EBPR performance. The laboratory scale treated 10L/day where 2 parallel treatment trains for milk and an equivalent carbohydrate mix as supplement to compare and study the P sequestration from hypothesised P ligands in milk and easily assimilable carbon (AOM) after fermentation for biological P uptake.

The aerobic bioreactors used submerged flat sheet membranes (AeMBR) to improve the effluent quality and reduce the suspended solid residues. The results suggested extra benefits from adding calcium chloride ($CaCl_2$) (200 ml at 250 mM/day or 200 mg/L treated) to form P complexes both in the anaerobic and aerobic zones (100 ml $CaCl_2$ 250mM/zone/day). To complete P removal a calcium phosphate ($CaPO_4$) further treatment stage (post membrane final effluent (F.E.)) was added for nucleation.

The combination of, A2O-N, exogenous carbon and calcium addition improved the performance of the EBPR, and enabled the laboratory units to achieve less than the 1 mg/L P required by the EU Directive. The process was tested at higher than normal P loads (maximum 100 mg/L) (domestic wastewater influent 15 mg/L). Experiments with influent P load ≤50mg/L, with 1% milk as AOM were compared to the carbohydrate mix and could remove soluble P to less than 1mg/L above 97% and less than 2 mg/L more than 99% of the in the time respectively. With an influent P load of 60mg/L (maximum 100 mg/L), the soluble P in the F.E. with milk was below 5 mg/L and below 8 mg/L with carbohydrates mix.

The results showed that most of the phosphorus was retained by the sludge during the anoxic-aerobic phases. The remaining phosphate in the F.E. was able to pass through AeMBR pore size (0.4 µm) and needed to be chelated by the nucleation process.

The results indicated this A2O-N modifications achieved stable nutrient removal and also offered the potential for more sustainable phosphorus recovery. The EBPR without AOM was 25% less efficient compared to milk and never achieved the E.U standard of 1mg/L in final effluent. The flat sheet membrane always achieved a NTU final effluent below 1 and the TOC always greater than 90% removal or less than the EU 125 standard regardless of the feeding COD/P ratio.

Keywords:

EBPR – COD/P – Exogenous source of carbon – Milk – Carbohydrates – Glucose – Calcium Chloride – Calcium Phosphate Nucleation – Crystallisation – A2O-IC – Membrane bioreactor – Modified University of Cape Town – Nutrient removal – Phosphorus removal – Dairy Wasterwater – Urban wastewater – Laboratory Continuous Flow

Anyone who has never made a mistake has never tried anything new.

(Albert Einstein)

Acknowledgments

This PhD studentship was funded by the Loughborough School of Civil & Building Engineering – Water engineering department

I am thankful to Mister Kennedy from Kubota Membrane Europe, United Kingdom for providing the membrane bioreactors and Loughborough wastewater treatment plant to allow.my access to their facilities.

I would like to thank Christine Barton for her indispensable help without which it would have been impossible to achieve my thesis, but also Jayshree Bhuptani who supervised me on a day to day basis and her immense dedication to keep up to date the apparatus and the water laboratory.

A special thanks to Geoffrey Russell for having to endure my avalanche of “Belgian” jokes, receiving weird gifts from Spain, and destroyed your favourite buckets. It was really great to have you there.

I am grateful to my supervisor Pr. Andrew D. Wheatley for his guidance and his support from my master to the end of my thesis

Lasts, but by no means least, I would like to thank my partner, Adriana Rubio and my mother Hernani Balmir for their unending encouragement, support and patience throughout my time working on this research

Nomenclature and abbreviations

List of acronyms

A2/O	-	Anaerobic/Anoxic/Oxic (aerobic)
ACP	-	Amorphous Calcium Phosphate
Alum	-	Hydrated aluminium sulphate
AeMBR	-	Aerobic Membrane Bioreactor
AnMBR	-	Anaerobic Membrane Bioreactor
AOM		Assimilible organic matter
APAO	-	Oxygen using Phosphate Accumulating Organisms
APHA	-	American Public Health Association
BOD	-	Biological Oxygen Demand
BPR	-	Biological Phosphorus Removal
CAS	-	Conventional Activated Sludge (Plants)
CIP	-	Cleaning-In-Place
COD	-	Chemical Oxygen Demand
COD:P:N	-	Chemical Oxygen Demand Ratio to Phosphorus to Nitrogen
CPR	-	Chemical Phosphorus removal
CF	-	Cross-Flow (Aeration)
DAF	-	Dissolved Air Flotation
DPAO	-	Denitrifying Phosphorus Accumulating Organisms
EBPR	-	Enhanced Biological Phosphorus Removal
EC	-	Electrical Conductivity
EPA	-	Environmental Protection Agency
EPS	-	Extracellular Polymeric Substance
EU	-	European Union
EUWFD	-	European Union Water Framework Directive
FISH	-	Fluorescence In Situ Hybridization
GAO	-	Glycogen Accumulating Organisms
HAc	-	Acetate
HAP	-	Hydroxyapatite / Hydroxylapatite

HPr	-	Propionate
HRT	-	Hydraulic Retention Time
IC	-	Ion Chromatograph
ICP	-	Inductively Coupled Plasma
µF	-	MicroFiltration
MBR	-	Membrane Bioreactor
MCP	-	Monocalcium Phosphate
mg/L	-	Milligram per Liter
MLSS	-	Mixed Liquor Suspended Solids
MUCT	-	Modified University of Cape Town
N	-	Nitrogen
NADH	-	Nicotinamide adenine dinucleotide
NTU	-	Nephelometric Turbidity Unit
O&M	-	Operation and Maintenance
OA	-	Oxygen Absorbed
OD	-	Oxygen Dissolved
OHO	-	Ordinary Heterotrophic Organisms
OrthoP	-	Orthophosphate
P	-	Phosphorus
PAC	-	Poly-Aluminium Chloride
PAO	-	Phosphorous Accumulator Organism
PHA	-	Poly-β-hydroxyalkanoates
PHB	-	Poly-β-hydroxybutyrate
p.e.	-	Population Equivalent
PO_4	-	Phosphate (orthophosphate)
PolyP	-	Polyphosphate
ppm	-	Parts per million
PHV	-	Poly-β-hydroxyvalerate
RAS	-	Returned Activated Sludge
rbCOD	-	Readily Biodegradable Chemical Oxygen Demand
RIS	-	Resistance In-Series model

RO	-	Reverse Osmosis
SA	-	Concentration of soluble fermentable substrates formed
SBCOD	-	Soluble biodegradable organic
SCOD	-	Soluble COD concentration
SBR	-	Sequencing Batch Reactor
scVFA	-	short-chain Volatile Fatty Acids
SF	-	Potential concentration of soluble fermentation products
SI	-	Concentration of inert substrates
SMP	-	Soluble Microbial Products
SNDPR	-	Simultaneous nitrification, denitrification, and P removal
SOUR	-	The specific oxygen uptake rate
SRT	-	Sludge Retention Time
SS	-	Suspended Solids
TMP	-	Trans Membrane Pressure
TOC	-	Total Organic Carbon
TotP	-	Total Phosphorus
TSS	-	Total Suspended Solids
UCT	-	University of Cape Town
UWWTD	-	Urban Waste Water Treatment Directive
VFA	-	Volatile Fatty Acids
VSS	-	Volatile Suspended Solids
WWTP	-	Waste Water Treatment Plant
WWTW	-	Waste Water Treatment Work

Glossary

Curd: The part of milk that coagulates when the milk sours or is treated with enzymes. Curd is used to make cheese.

Pasteurized milk: Milk free from harmful bacteria (flash heat treated to kill active bacteria) as sold to general public

Raw milk: Milk as collected from the farm, prior to any treatment.

Ultra-high temperature products: Any liquid milk products that have been treated by the ultra-high temperature process and packed aseptically.

Whey: Liquid left once curd has coagulated.

Industrial wastewater: Any waste water which is discharged from premises used for carrying on any trade or industry, other than domestic waste water and run-off rain water

Urban wastewater: Domestic waste water or the mixture of domestic waste water with industrial waste water and/or run-off rain water.

Domestic wastewater: Waste water from residential settlements and services which originates predominantly from the human metabolism and from household activities.

List of Technologies

Table of Contents

List of Tables

List of Figures

1.0 Introduction

Demographers project that the world's population will increase from 6.8 billion to 8.3 billion by 2030 (Eberstadt, 2012). The National Intelligence Council (NIC) (2012) predicted that the total demand for energy and fresh water will increase by 35% in the next 20 years as a result of more agriculture and more industry to satisfy the minimum expectations from this growth. Finally, the world's climate will continue to change in ways to endanger food production and dislocate people. Black *et al.,* (2011), stated that these environmental changes will lead to major disruption that will affect both developing countries but also the developed countries.

The sustainability message is now more integrated and the vast majority of the people appreciate the message. However, sustainability is between, a nice thing to do (e.g., integrated the wind mills in the landscape), and a must do (e.g., reduce the carbon footprint or recycle the water). Water recycling from wastewater treatment must increase drastically, and to do this more efficiently it requires further research on nutrient recovery, such as N and P, in order to preserve the clean water and source of fertilizer.

The eutrophication of water caused by the excess of nutrients can be dramatic with economic consequences (e.g.: reduction in species diversity and loss of fisheries). The treatment combination, of biological and chemicals, used now to remove the phosphorus from wastewaters is working well, and the Water Framework Directive (2000/60/EC) (WFD) provides significant driving force to improve the water quality by 2015. Depending upon the Area Sensitivity to P, designated by the Environment Agency (EA), the Urban Waste Water Treatment Directive (Annex I) (UWWTD) includes two different standards according to the size of wastewater treatment plant. For population equivalents (p.e.) of 10 000 – 100 000 the maximum is 2 mg/l in the discharge and/or 80% removal, if the p.e. is greater than 100 000 then the maximum is 1mg/l in the discharge and/or 80% removal. Unfortunately, water companies believe that the WFD implementation will have significant implications for future investment (OFWAT/WFD/003A) including problems with the current recycling of waste products (i.e., producing sludge containing the coagulant such as iron or aluminium) and thus costs for the waste management. Moreover the UK Technical Advisory Group has estimated that, in England, approximately 64% of rivers and 77% of lakes are at risk of failing the proposed WFD phosphate standard for good status. The costs of coagulants, the most common P removal technology in Europe, are also expected to continue to increase both because of the loss steelmaking abroad and competition for these chemicals .

Thus there is a need for a sustainable, cheaper solution, to P recovery and this is the topic of this thesis. Usually companies are reluctant to implement new technologies because of the need for complete dependability. Many new approaches have been proposed by research but most are rejected as a consequence of the cost/benefit analysis. Biological treatment systems are the most sustainable since they are based on natural microbiological processes at normal temperature and pressure. Higher degradation activity, and thus removal, is improved by promoting increased bacterial concentrations that has recently been possible through the use of membrane technologies.

Membrane bioreactors have been identified as one of the robust technologies for water reuse in the future (Le-Clech *et al.,* 2006; 2010). The effects of combinations of EBPR and coagulants on membrane have not been well researched. These need to be defined further to ascertain their applicability within sustainability criteria for wastewater treatment (e.g., KWh consumption and residues produced) (Alvarez-Vazquez *et al.,* 2004)

However, in case of P removal the desired type of P accumulating bacteria are in competition with others and must therefore be increased in the wastewater treatment processes. This is why the designs and operations are still subjects of development and research. In UK the wastewaters are diluted with storm waters and this reduces the COD concentration required by the EBPR bacteria. Unfortunately the EBPR bacteria are also slow growing. Their metabolic selection to promote their growth and retention is encouraged by successive stages of different redox environments, these require, providing adequate soluble assimilable substrates. This also is investigated in this thesis.

2.0 Literature review

2.1 Water availability and demand

2.1.1 Water availability

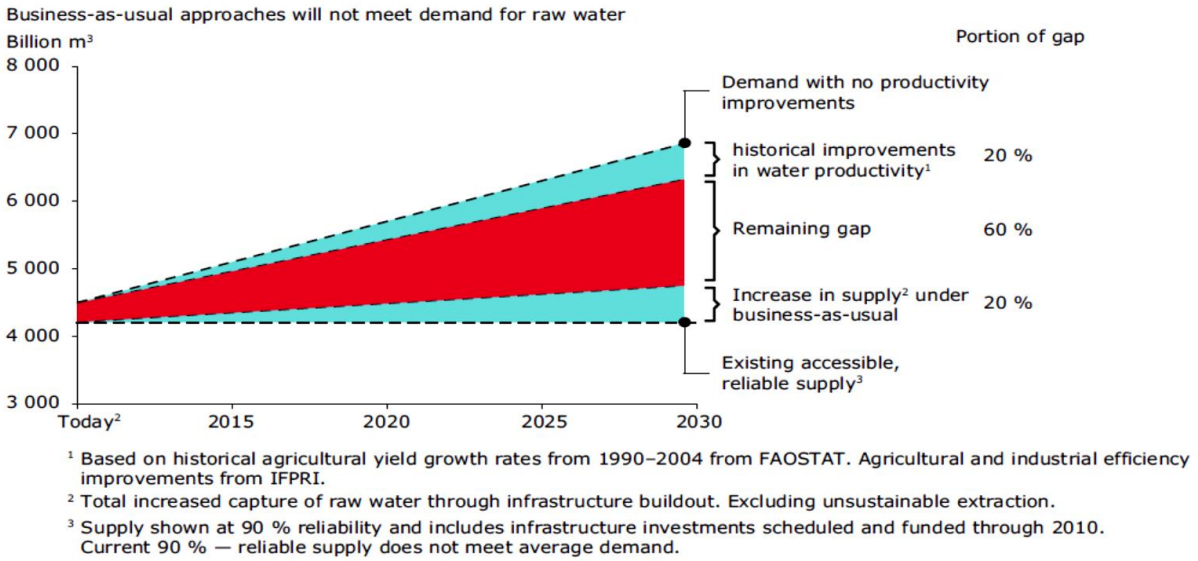
Water on earth is an abundant resource with a natural cycle, clean and unpolluted water is essential for many living organisms, among them are humans and in particular urban populations. In the recent years, there has been increasing awareness of the need to protect the world water supplies which are also fundamental to agriculture. World population growth has introduced water scarcity, because of the uneven technology and distribution of water availability in time and space, clean fresh water in Northern countries is not yet a pressing matter. However, the occurrence of seasonal or longer term droughts, water scarcity situations have also become noticeable in Europe (Dworak *et al*., 2007) and is worrying for more than two billion people living in highly water stressed areas (e.g., arid and semi-regions, highly urbanised areas particularly susceptible to water stress) (Oki *et al*., 2006). Climate change, pollution and over-usage are the most threatening factors that jeopardise the available world supplies (Elizondo, 2010).

As populations grow there has to be a shift to becoming more urbanised, and some regional clusters are reaching the upper limits of the available water resources because most individual houses have facilities such as flushing toilets, washing machines, bath and showers. The pressing needs for socio-demographic, economic and technological changes in developing countries are creating new clusters (Moss *et al.,* 2010). These needs are still satisfied by providing more infrastructures and associated major storage facilities with dams for hydropower and canalisation for inland aqueducts which have reduced connectivity and the quantitative status of natural water bodies (Kristensen *et al.,* 2010). Therefore, the extent of water scarcity and drought will worsen with impact on the economy, society and the environment (Dworak *et al.*, 2007).

Water demand in the European Union (EU 27) is around 247 billion m^3/year, where energy production is using 44%, agriculture 24%, public water supply (includes households, public sector and small businesses) 17% and industry 15 % (Dworak *et al.*, 2007). A use of between 50 m^3 and 150 m^3 per year per capita for household in Western Europe has been measured. In the United Kingdom demand is at 97 m^3 per year per capita on average (Eurostat, 2012).

An integrated approach to water resource management is a real challenge for authorities to respond to the stress in demand and quality, in a sustainable way for an acceptable price.
See in Figure 1 a forecast and management scheme based on historical data made by Kristensen et al. (2010). The EU Water Framework Directive (WFD) for 2015, and the EU 2020 Strategy from the European Commission's 'Blueprint for safeguarding European waters' give the targeted objectives to reach within Western Europe. Other policy changes in economic strategy can help, for example, the supervision in agricultural and industrial use, water-savings campaigns by increasing efficiency in households, water loss controls, water reuse and recycling.

Figure 1: Management to meet the forecasting demand

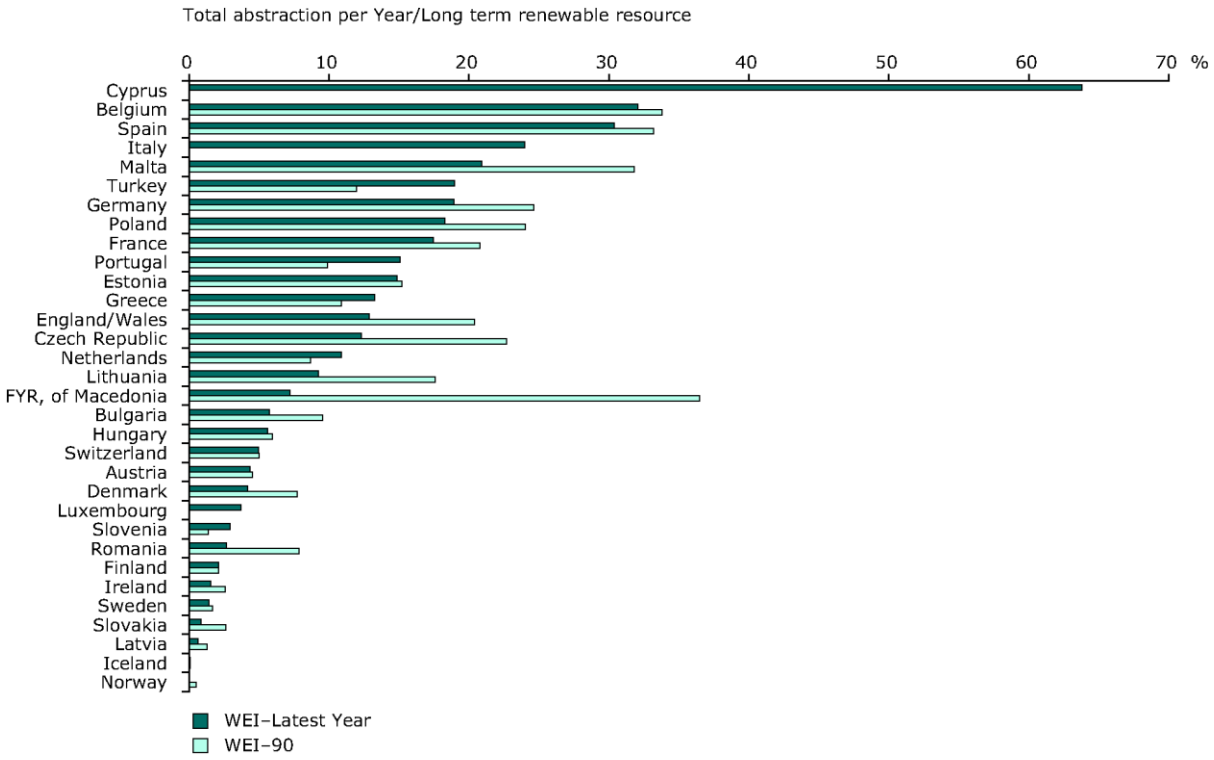


Source: 2030 Water Resource Group, 2009. (Kristensen *et al.,* 2010)

Some changes in water availability are creating conflict between authorities at both national and international levels (Cooley *et al.,* 2009). Water is very critical for the production of food in the country and to sustain the domestic demand. Conflicts over water rights have historically been common (Strzepek & Boehlert, 2010). Water management is in many arid countries a challenging policy, especially in critical situation where the growing population and expectations are generating political pressures and highlighting shortcomings in infrastructure (Engle *et al.,* 2011). Further unexpected problems such as climate change or other natural disaster could lead to political instabilities (Engle *et al.,* 2011). Therefore, it is obvious that for some countries water can be a key control for a greater independence to foreign countries as the uncertainties of a specific system can change persistently in an uncontrollable way (Engle *et al.,* 2011).

The Water Exploitation Index (WEI) defined as the mean of total fresh water abstraction divided by the mean annual total renewable freshwater resource at the country level (expressed in %). WEI is a good national indicator of the pressure on freshwater resources where a WEI above 20% indicates a water source under stress, and more than 40% implies unsustainable use and severe stress of water resource (Raskin *et al.,* 1997). However national estimates of WEI for a country may obscure some region differences that can suffer severe stress demand. For example, for the whole Spain the average WEI is 30% as seen in Figure 2, but regionally it can exceed 100% capacity as measured in south-eastern river basins of Andalusia and Segura. Demand in this region can only met by transfers from other river basins, water reuse and desalination (Kristensen *et al.,* 2010).

Figure 2: European Water Exploitation Index



Note: Annual total water abstraction as a percentage of available long-term freshwater resources around 1990 (WEI-90) compared to latest year available (1998–2007) (WEI-Latest Year).

Source: EEA-CSI 018 - http://www.eea.europa.eu/data-and-maps/figures/water-exploitation-index-wei-3/signals-cc-mitigation-fig-1-ny.eps/image_original, (last modified Sept 05, 2011) (Accessed 19 October 2012)

2.1.2 Phosphorus availability

The importance of phosphorus in human biology and industry make that this product can be found in excess in the environment creating problems in water surfaces reducing its availability. However, phosphorus is also a valuable product and wastewater treatments plants must adapt their technologies to overcome the new challenges explained in this literature review.

2.1.2.1 Phosphate supply

Phosphate rock mines are located outside the EU. The majority of phosphate rock reserves are located in the Western Sahara and Morocco. The other major holders of phosphate rocks reserves are, from the larger to the smaller: Iraq, China, Algeria, Syria, Jordan, South Africa, the USA and Russia. The limited domestic phosphate rock reserves in Europe are located in France, Germany, Italy, Spain and UK. Three quarter of the mineral P is used for fertiliser manufacture. Possible new reserves could be uncovered if the rising price can sustain that new explorations. However the new places discovered so far will remain outside the EU (e.g., Saudi-Arabia, coasts of New Zealand (offshore) and Namibia) (De Ridder *et al.,* 2012). The United States Geological Survey (USGS) estimated the production of phosphate rock at 181 million metric tons (mmt) in 2010 and 191 mmt in 2011. Over two thirds of the production was from the USA, China and Morocco (Western Sahara included). According to the International Fertilizer Association, the EU exported 62,000 metric tonnes of phosphate rock and imported 7,518,000 in 2010. (De Ridder *et al.,* 2012).

2.1.2.2 Phosphate usage

Phosphorus, together with nitrogen and potassium, are the key elements for world agricultural production feeding (raise crops, feeding animals…) (Cordell *et al.,* 2009). Phosphorus is also an element present in all living things and is crucial for cell growth (i.e., membranes and nucleic acids) and cell energy production through the adenosine triphosphate (ATP) (de Ridder *et al.,* 2012).

Other usage of phosphorus is very wide such as listed below Bala Suresh & Yoshio Inoguchi, (2005):

- Fireworks / matches;
- Weapons;
- Lubricating oil additives;
- Fertilizers;
- Pesticides;
- Surfactants and sequestrants;
- Miscellaneous chemicals and alloys;

- detergents:
    - Water softener: Na_3PO_4 - $Na_4P_2O_7$ - $Na_5P_3O_{10;}$
    - As cleaner: $(KPO_3)x$ - $K_4P_2O_7.3H_2O$;
    - Bath salts: $Na_4P_2O_{7;}$
- toothpaste ;
- Food:
    - acid food ((ortho)phosphoric acid (E 338));
    - antioxidant (in cola);
    - Use as buffer ((E339-1/2/3) – potassium phosphate (E340));
    - Agent to avoid coagulation e.g. in salt as $MgHPO_{4..}$

Typically about half the P in domestic wastewater is from metabolism and about half from household products.

Bala, Suresh & Inoguchi (2005) have found that the demand for P has reduced because of environmental restrictions on the use of phosphates (particularly in laundry detergents) and competition from more efficient less costly purified wet-process phosphoric acid. The reduction came mainly from more efficient fertiliser use in Europe and North America, but also Japan that has also stopped completely the production of yellow phosphorus due to high electric power costs. Russia has also reduced consumption primarily because of their economic problems and reduction in demand from the larger Soviet Union. On the other hand, China has considerably increased its production and usage, its exports (including its derivatives and products) impacting the world market. Between 1999 and 2010 South Asia's imports doubled, whereas the EU reduced imports by 30%. These applications lead to phosphorus in different media and the control and treatment needs to be different as well. The stability of phosphorus when it is bound with different elements may also require a wide range of different treatment process.

2.1.2.3 Phosphorus recovery

Phosphorus is a finite resource and the known reserve of phosphorus rock in the world is depleting rapidly because unlike the nitrogen (nitrogen cycle), phosphorus is not a renewable element, and the world demand is increasing because of the population increase (Cordell *et al.,* 2009). Moreover, no substitute is known to be able to replace phosphorus in living organisms and agricultural soils need to be enriched by phosphate-derived fertilizers since plants and rain deplete soils of P element (de Ridder *et al.*, 2012).

Although there are environmental restrictions on the use of phosphates, good strategies such as recovering P from sewage sludge and other waste materials containing P will be important to improve the sustainability of the P agriculture cycle (van Vuuren *et al.,* 2010). For example, over the period 2005 – 2007 on average only slightly more than 40% (approximately 0.12 mmt of phosphorus) of sewage sludge generated within the EU 15 was reused in agriculture the remaining 60% was wasted (de Ridder *et al.*, 2012).

Phosphorus recovery from wastewaters using alternative chelating agents, is in the industry interest and to recover phosphorus as calcium phosphates is advantageous since there will be no restrictions on direct use in agriculture rather than, for example, with iron or aluminium (Driver *et al.,* 1999). Calcium phosphate is the main component of phosphate rock used as a fertilizer and moreover calcium phosphates are also the most common form in the natural aquatic environment (Golubev *et al.,* 1999). The necessity to recover phosphorus is important for two main risks, the political-economic risks and eutrophication of the environment.

2.1.2.3.1 The political-economic risks

The EU is almost entirely dependent on supplies of P from the rest of the world where there is a growing demand and limited supply which will cause phosphate rock prices to rise. Previsions made by the Global Phosphorus Research Initiative GPRI) calculated that the peak production might be reached in 2033 (Cordell *et al.,* 2009). Other studies have suggested that in 2100 half of the available phosphorus resources will be depleted (Van Vuuren *et al.,* 2010). China and USA despite being large producers, they export nothing because of their own consumption (de Ridder *et al.,* 2012).

The price of phosphate rock, based on World Trade Organisation (WTO), was below 50 $/mt (metric ton) until 2008 where the price peaked rapidly to around 350 $/mt, then reduced in 2009 to about 125 $/mt to remain higher than before 2008. The price increased again at close to 200 $/mt in 2011 to reduce slowly and stabilise at 110 $/mt in 2014. Therefore most countries rely exclusively on imports from mostly unstable countries (e.g., in Africa). Technical issues (i.e., waste, spillage, over-use) or poor infrastructure which raises international concern on environmental impact which, for example, restricts Russian exports from international control.

Other risks can be listed such as:

- Competition to secure supply;
- Vertical integration (final products move up the value-added chain);
- Protectionist measures (trade restriction).

2.2 Water reuse

In regions that are subjected to water stress, achieving high quality treated effluents can allow water reuse and recycling providing a contribution to water security and avoid abstraction from natural water bodies. The challenge for the wastewater treatment plants, taking the stringent regulations in account, is to offer viable and competitive water resource possibility.

Typical reuse applications (EPA, 2004)

- Urban reuse;
- Industrial Reuse;
    - Cooling water;
    - Boiler make-up water;
    - Industrial process water (paper industries, chemical industries …)
- Agricultural reuse (nutrient enrichment);
- Environmental and recreational reuse;
- Groundwater recharge;
- Increasing potable supplies.

2.2.1 Water reuse benefits

The two biggest advantages of water reuse are first, the improvement in the quality of wastewater discharges into the environment through better control of the pollution and, as in the case of this thesis, nutrients (phosphorus and nitrogen). These nutrients are valuable in the agricultural sector reducing the fertilizer application (Molinos-Senante, 2011; Aquarec, 2006). Water quality is better for fauna and flora but also several other beneficiaries downstream (Aquarec, 2006).

The second big advantage is to increase the water availability supply and potentially with less energy consumption. For example actual processes (e.g., settlings, sand filtration…) are less expensive to operate compared to desalination and schemes for the mass transfer of water such as use of membranes. Recycled treatment effluent can often be used directly at the point of production (e.g., the city reservoir save energy by reducing the pumping distance to the point of usage) for recreational, toilet flushing and cleaning. Additional stages of treatment are needed for potable use (Aquarec, 2006).

2.2.2 Drawbacks

Despite the important advantages offered by the recycling water, the program is facing some difficulties. These are in two groups, perception and persistent quality issues. Implementing regulations with high standards for micro-quality and persistent pollutants (e.g. pesticides) can be very hard to achieve. Social acceptance from the population is also a difficulty that comes mainly from, drinking treated wastewater, but also from a low level of awareness concerning recycling issues (Jimenez and Asano, 2008). There is a strong adverse public reaction to possible negative impacts, for example affecting soils, plants and aquifers by using new technologies such as the hydraulic fracturing (or fracking) to recover natural gas from shale rock.

The difficulty of demonstrating the economic benefits, and therefore the financial performance of water reuse, is reducing the potential acceptance for adopting and changing practices for non-conventional water resource (Jimenez and Asano, 2008). Generally, the external impacts are neglected in cost assessments, as it is in the majority of cases only internal costs are taken in account, the economic impact difference becomes narrower (Molinos-Senante *et al.,* 2011).

2.2.3 Risks

Risk considerations for human health and environment should always be considered before applying for water reuse project. There are potential risks in reclaimed water in agricultural irrigation. As noted, the risks are from pathogens and trace quantity of persistent pollutants (heavy metals, organic matter and chemicals).

Water reuse can also increase flow to the rivers or capacity to lakes in arid and semiarid regions as water bodies flow can be improved thanks to the treated wastewater discharge, and reduce the water withdrawn (Aquarec, 2006). Good effluent quality discharge must be ensured as it could influence natural ecology and biodiversity downstream. Therefore it is important to identify the hazard and assess the exposure in order to characterize the potential risks and benefits.

Nutrients N and P in wastewater, the subject of this thesis are an impediment to greater water recycling and this is discussed in more detail in the next section

2.2.4. Eutrophication

The eutrophication is the growth and proliferation of organisms, mainly algae that threaten biodiversity within natural water. Further problems occur when the algae population die, their decomposition consumes all the oxygen in the water, leading to anoxic and anaerobic conditions potentially releasing anaerobic toxins. The environmental deterioration caused by eutrophication has become a priority for the E.U. water quality objectives because biodiversity is already seriously affected.

The increase of nutrients into surface waters are mainly caused by phosphorus (P) but also nitrogen (N) compounds. Worldwide Increasing awareness of this causative effect on eutrophication has led to the introduction of legislation controlling discharge of P to receiving waters.

In order to avoid a continuous and further degradation of the natural waterways, authorities in charge of the environment in UK such as Environment Agency (E.A.) and the European Union (E.U.) have increased the restrictive regulations controlling the release of nutrients in treated wastewaters. These regulations have led to the need for more efficient technologies for nutrient removal. For example policies by the EU are the Directives on Bathing Water (76/160/EEC), Sewage Sludge (86/278/EEC), Urban Waste Water Treatment (91/271/EEC), Nitrates (911/676/EEC), and on Integrated Pollution Prevention Control (96/61/EEC), all of which are now part of the EU Water Framework Directive (Schröder *et al*., 2010).

The use of phosphate fertilizer in the agricultural sector is an important source of phosphate in surface waters. In addition studies have indicated that eutrophication can be controlled by reducing the phosphorus (P) load from wastewater discharges (Dillon and Molot, 1996). The combined EU urban wastewater contains about 1.14 mmt as P_2O_5 (di-Phosphorus penta-oxide) per year. This is equal to about 34% of imported phosphate (3.4 mmt P_2O_5) in 2011 in the EU. Moreover, the correlation between the phosphate concentration in surface waters and eutrophication creates extra costs and difficulties for drinking water purification (de Ridder *et al.,* 2012).

2.3 Urban Wastewater

2.3.1 Introduction

Urban wastewater is a mixture from households and industries effluents and surface runoff. Pollution from industrial effluent discharge will be related the type of production and if it is hazardous (EU REACH), then the industry would not be connected to the sewer.
Urban runoff pollution will be impacted more by traffic and by the catchment area for example antecedent dry period. Types of pollutants would include metals and persistent organics as well as the usual carbon and nitrogen.

Household wastewater production is divided in two categories (Palmquist *et al.*, 2005; van Voorthuizen *et al.,* 2008):

- Greywater, representing between 70-75% is the effluent from bathing and showering, and kitchen sink, laundry and dishwasher;
- Blackwater, between 25-30% is made of waste from the water closet, containing faeces, urine, toilet paper and flush-water.

2.3.2 Urban wastewater characterisation

Increasing the knowledge of grey- and blackwater characteristics is important to understand the relative contribution of priority recoverable pollutants from urban wastewater. This information is useful for the management of both traditional and alternative wastewater systems as the objectives change to agricultural demand and climate change priorities (Palmquist *et al.*, 2005). In order to have an efficient management and more adaptive wastewater treatment plant, the design and planning infrastructure must take in account the external environment uncertainties (Domiguez *et al.,* 2006; Aulinas *et al.,* 2011).
Climate change and increasing urbanisation are bringing new challenges but the complexity and the dynamics of change in wastewater make the predictions impossible (Astaraie-Imani *et al.,* 2012).

For this study, urban wastewater will be divided into three categories:

1. It is the majority component of the influent wastewater entering the wastewater treatment work (WWTW).
2. Industry wastewaters entering or not in the WWTW system are usually specific to the catchment area. Example food processing wastewaters can be considered beneficial to improve P removal by entering in treated wastewater.
3. Wastewater entering the WWTW in UK is from combined sewer network which include, the highly fluctuating, storm water flows from which only water from primarily run-off from roofs and streets.

2.3.3 Fraction composition of wastewater

Many studies agree on the difficulties on establishing a standard characterisation of grey and black water (van Voorthuizen *et al.,* 2008; Palmquist *et al*., 2005) because the sources vary according to the standard of living (e.g. the washing practices, the food) and demography.

A report has been made by Jonsson *et al.* (2005) based on literature review and previous work from Swedish households and developed the parameters for this thesis and research. The report has been called the URWARE model. Swedish lifestyle and food is about the same as found in the other EU 27, and it was considered acceptable to take these figures as basis for United Kingdom. Table 1 presents the fraction of European domestic wastewater.

Notation and parameters
TS: Total Solids, ash = TS-VS;
TSS: Total Suspended Solids
VS: Volatile Solids
COD_{Tot}: Total COD = (1)+(2)+(3)+(4)
$COD_{Sol,Bio}$: Soluble and biodegradable (1)
$COD_{sol,In}$: Soluble and inert (2)
$COD_{Part,Bio}$: Particulate and biodegradable (3)
$COD_{Part,In}$: Particulate and inert (4)
N_{Tot}: Total Nitrogen
N_{NH3NH4}: Ammonia/ammonium-nitrogen
N_{NO3}: Nitrate-Nitrogen
$N_{Sol,Org}$: Nitrogen, soluble & organic
$N_{Part,Org}$: Nitrogen, particulate & organic
P_{Tot}: Total phosphorus = (5)+(6)
P_{PO4}: Phosphorus-phosphate (5)
P_{Part}: Particulate phosphorus (6)

Table 1: URWARE household wastewater, summation of urine, faeces and grey water, expressed as grams/person/day (g p^{-1} d^{-1}), (Jonsson *et al.*, 2005)

Parameter	Urine	Faeces & toilet paper	Greywater total	Household WW total society
H_2O	1487	110.7		
TS	**20**	**53.4**	**71.2**	**144.3**
TSS	0.76	48.0	17.6	66.36
VS	7.4	46.4	41.6	95.40
COD_{tot}	**8.5**	**64.1**	**62.4**	**135.0**
$COD_{sol,bio}$	7.23	5.2	24.7	37.13
$COD_{sol,in}$	0.67	0.4	1.3	2.37
$COD_{part,bio}$	0.46	47.2	27.4	75.06
$COD_{part,in}$	0.14	11.3	9.0	20.44
BOD_7	5.0	34.1	33.8	72.90
N_{tot}	**11.0**	**1.5**	**1.53**	**14.03**
$N_{NH3/NH4}$	10.3	0.3	0.25	10.85
N_{NO3}	0	0	0.01	0.01
$N_{sol,org}$	0.6	0.45	0.47	1.52
$N_{part,org}$	0.1	0.75	0.80	1.65
P_{tot}	**0.9**	**0.5**	**0.68**	**2.08**
P_{PO4}	0.81	0.1	0.29	1.2
P_{part}	0.09	0.4	0.39	0.88

The concentrations of different components represent what are normally measured at wastewater treatment plants. The concentration value for each variable is based on the assumption that the wastewater flow is 200 L $pe^{-1}day^{-1}$ (equivalent to 73 m^3 $year^{-1}$).
This figure is relatively high compared to the UK where the range without storm or industrial wastewater is about 150 L $pe^{-1}day^{-1}$ (DEFRA, 2012).

Composition measurement of urine and faeces with toilet paper (Jonsson *et al.,* 2005):
Values are expressed as g pe^{-1} day^{-1}. Normally 55 - 70% of the time is spent at home and the corresponding proportion of the excretion is collected at home. The amounts collected in different measurements have therefore been extrapolated to the excretion during 24 hours. The composition is that expected after transport in a sewage system.

Composition measurement of greywater and biowaste (Jonsson *et al,* 2005):
Flow per person per day (pe^{-1} day^{-1}) and composition of greywater expressed as g pe^{-1} day^{-1}, normally only 55-70% of the time of a person is expected to be spent at home. A volume of 30% was added to the greywater corresponding to the greywater generated when the person is not at home. Table 2 expresses the data report from 6 major Swedish WWTP (Bromma (1year report), Henriksdal (1year report), Käppala (3years report), Rya (3years report), Syvab (3years report) and values measured at Båtbryggaregatan). The WWTPs of Stockholm are compared to other URWARE values according to Table 1, (Jonsson *et al.,* 2005).

Table 2: Relations between COD_{tot}, BOD_7, N and P influent expressed as kg $pe^{-1}year^{-1}$

WWTP Mix of 5 major Swedish WWTP	BOD_7/COD	COD/P	BOD_7/P	N/P
Minimum of above WWTP	0.31	64.2	23.0	5.4
Maximum of above WWTP	0.51	83.3	42.8	7.2
Median of above WWTP	0.46	68.0	31.5	5.8
Båtbryggaregatan, from averages	0.46	58.8	27.1	5.5
Båtbryggaregatan, from medians	0.49	57.1	27.8	5.8
URWARE, household ww	0.54	64.9	35.0	6.7
URWARE, household – 20% BOD_7	0.48	57.9	28.0	6.7

If it is assumed that 20% of BOD_7 is degraded on the way to the WWTP therefore URWARE-20% ratios is more in accordance with measured data in Table 1. The BOD_7/COD ratio concurs with the measurements from Båtbryggaregatan (Magnusson, 2003; Jonsson *et al,* 2005) and is close to the median from wastewater treatment plants. In particular the BOD_7:P ratio measured at Båtbryggaregatan are within 1% to the URWARE calculated, but includes some industrial wastewater and some stormwater.

The URWARE measured flow mainly consists of household wastewater at the inlet of the wastewater plants, and it is a little lower than the median for URWARE wastewater measurements (Table 1) (Jonsson *et al,* 2005).

2.4 Monitoring wastewater treatment plant process

2.4.1 Influent changes

Wide fluctuations in substrate and in particular excess substrate can be very detrimental for the biological activities. Primary treatment, screens and settlement are designed to reduce the wide range of feed, it is very difficult for the WWTP to stop or prevent toxic agents from entering the system. Historical precedents flow control and prior indications (e.g., weather forecasts) can give enough time to take proactive measures and avoid negative event from occurring (Archibald *et al.,* 2001; Ren, 2004).

2.4.2 Biomass in wastewater.

Biological wastewater treatment processes rely on microorganisms to break down and assimilate organic compounds. This mechanism occurs naturally but in order to accelerate and optimize it, the bacteria are grown in bioreactor conditions where the systems engineered increase the biodegradability rate with a higher biological concentration (Archibald *et al.,* 2001).

1. Biomass activity.

Simple methods are used to identify microbiology activity such as total suspended solids (TSS) but, the method measures only the mass giving little information about activity. Respiration activity measured by the 5-days biological oxygen demand is a very long time to make the analysis and take appropriate measures to improve biological responses. Respirometric activity can be obtained over a shorter period of time to give a method for biological early warning (BEW) by measuring the bioreactor consumption of dissolved oxygen (DO) (Archibald *et al.,* 2001). The method used is called the specific oxygen uptake rate (SOUR), it measures the oxygen uptake rate (OUR) per unit of dry biomass. However, according to some research SOUR gives poor indications in sludge health or system capacity (Archibald *et al.,* 2001, Arslan-Alaton *et al.,* 2005). In any event, biological system monitoring requires the input of other parameters measured daily such as total suspended solids (TSS) and volatile suspended solids (VSS), total organic carbon (TOC) or chemical oxygen demand (COD), pH and dissolved oxygen (Oller *et al.,* 2011).

2. Factors inhibiting the biomass activity.

Temperature, variations in BOD loading, the pH and toxic compounds are the principal factors in reducing efficiency. The impact can be a deficit of oxygen or reduction in substrate removal, and the microbial community becoming unviable (Latorre *et al.,* 2007). Efficiency can be reduced because of the nature of components in the sewage such as many organic substances produced by chemical industries as household products are toxic or resistant to biological treatment (Oller *et al.,* 2011). Natural elements like metals, metal oxides and metal salts can be toxic to microorganisms (Oller *et al.,* 2011). For example free cyanide above 0.2 mg/l, Ethylbenzene, Chlorobenzene, Trichloroethylene, Zn^{2+} and Cu^{2+} as heavy metals and phenol above 200 mg/l, all inhibit nitrification (Kim *et al.,* 2008; Juliastuti *et al.,* 2003).

Thus wastewater characteristics are very variable making consistent removal or recovery of specific components such as P quite difficult. Some of the problems encountered with existing P removal processes are described in the next Section.

2.5 Phosphorus removal processes

The most common mineral form of phosphorus is apatite, which is a calcium phosphate with variable amount of OH^-, Cl^- and F^- (hydroxyl-, chloro-, or fluoro-), and some other phosphate minerals contain aluminium and/or iron (Maurer and Boller, 1999). Because of their low solubility it may be considered that most problematical of the phosphorus present in wastewater is in soluble form. Therefore, the transformation of soluble phosphorus into solid phase is needed either chemically or biologically, to help meet the EUWWTD.

The transfer to a solid phase may be performed in the following ways (Balmér & Hultman, 1988; Maurer & Boller, 1999; Tchobanoglous *et al.,* 2003):

- Chemical precipitation and adsorption of phosphorus by trivalent metal salt addition, the most common are aluminium sulphate, ferric chloride, combination of ferrous (bivalent) and ferric sulfates and calcium salt (lime);
- Biological in two general ways:
    - Uptake due to nonexchangeable phosphorus or/and
    - Enhanced uptake by bacteria
- Ion exchange and adsorption

Three groups of phosphorus compounds are important in wastewater:

- Organic phosphates: the microbial decomposition in wastewater releases P into smaller organically and/or chemically to orthophosphates;
- Condensed inorganic phosphates represent the polyphosphates (component of detergents, softener…) and the metaphosphates. Depending on the product they may contain 2 to 7 P atoms;
- Inorganic orthophosphates PO_4^{3-} ions as H_3PO_4, $H_2PO_4^-$, $H_2PO_4^{2-}$ and PO_4^{3-} depending on pH. The most prevailing species in wastewater at neutral pH are the $H_2PO_4^-$ and $H_2PO_4^{2-}$.

2.5.1 Historical development

Domestic and industrial wastewaters are already commonly treated by using chemical processes such as alum and/or ferric coagulation to remove suspended solids and nutrients (P and N) discharge (Jenkins *et al.,* 1971). This treatment is generally at least preceded by screening and grit removal to enhance the precipitation performance of phosphorus and improve solids settling in sedimentation, reduce the polyelectrolyte coagulant consumption for sludge thickening and help the elimination of hydrogen sulphide in sludge digesters (ICON, 2001). Chemical phosphorus removal from wastewater is increasingly practised, and often more common in the final settlement tank, after biological treatment and before any discharge into water courses.

Final adjustable chemical dosing ensures meeting the standard, but also reducing the chemical demand at the cleanest part of the process. Jenkins *et al.* (1971) pointed out that iron or aluminium (with or without lime) were used to remove BOD and suspended solids as the earliest type of wastewater treatment in the last century (about 1900) and were also used to improve the settling characteristics of activated sludge almost as soon as it was introduced in 1914..

Problems arose with chemical treatments which still are relevant: the cost, the sludge quality and quantity and making sludge disposal problems worse as a result of the extra metals (Yeoman *et al.,* 1988; Tchobanoglous *et al.* 2003). Biological treatment processes have therefore always been preferred except in cold or warm climates. However chemical treatment for phosphorus removal from wastewater was reintroduced in the late 1960s and 1970s, when concern over eutrophication started.

Implementation of chemical dosing (mainly with iron salts, but also aluminium) for P removal could be retrofitted to existing plants and was extensively used in the USA (particularly in the Great Lakes of North America), Canada and parts of Europe (Scandinavia and Switzerland) (D'Elia & Isolati, 1992). Chemical precipitation also enhances the removal of other but potentially toxic elements from sewage effluent by increasing the transfer of metals to sewage sludge.For example, Cr precipitation with aluminium sulphate can be concentrated by a factor of three in the sludge produced.

The increase in removal of Cu and Zn precipitation can be 50% compared to sedimentation without addition of aluminium. However, iron-based precipitant used in wastewater treatment are usually derived from industrial by-products, for example after the production of titanium oxide. Potential undesirable effects on the metal content of sludge could provide a significant barrier to recycling due to the trace concentration of potential toxic elements (ICON, 2001).

2.5.2 Mixing Options

De Haas *et al.* (2000) claimed that chemical addition can take place at one of three stages of wastewater treatment:

- Primary treatment (i.e., primary sedimentation, if present), immediately before settlement for which the term "pre-precipitation" may be used.
- Secondary treatment (typically into an activated sludge or bio-filter system) for which the term co-precipitation may be used.
- Tertiary treatment (chemical flocculation followed by sedimentation or flotation, sometimes followed by filtration) for which the term of "post-precipitation" or separate may be used.

2.5.3 Iron Salts

For chemical P removal, or in a combination of chemical and biological removal, iron salts are still the most widely used. The main reason is to lower the water toxicity, De Haas *et al.* (2000) stated that both iron species (Fe^{2+} / Fe^{3+}) combine with orthoP, and hydroxide in competing precipitations reactions. The iron hydroxide also participates in the removal of phosphate by adsorption and complexation and thus creates a slower exchange reaction with orthophosphate (orthoP) ions.

From stoichiometry, ferric (Fe^{3+}) ions form $Fe(PO_4)_3$ (strengite) in the reaction with orthoP, while ferrous (Fe^{2+}) ions form $Fe_3(PO_4)_2.8H_2O$ (vivianite). Both ferric and ferrous ions also react with hydroxide to form amorphous iron hydroxide flocs. The iron hydroxide can destabilise the negatively charged iron phosphate colloids, enmesh them and provide an adsorption capability for orthoP and polyphosphate (polyP) molecules (e.g. pyrophosphate and triphosphate) which are commonly used as softeners in detergents (De Haas *et al.* 2000).The stoichiometric mass ratio of Fe:P for $FePO_4$ (ferric) and $Fe_3(PO_4)_2$ (ferrous) is 1.8:1 and 2.7:1, respectively (De Haas *et al.* 2000). Jenkins *et al.* (1971) however reported that excess ferric iron is necessary to precipitate the phosphate because of the competition mainly from hydroxyl ions but also with other salts and the need to destabilise colloids.

Of the iron salts, iron (III) chloride (ferric chloride) is most commonly used for P precipitation, at WWTP mainly to avoid adding sulphate potentially leading to more corrosive and malodorous sulphides.

Yeoman *et al.* (1988) quoted by De Haas *et al.* (2000) assumed that under aerobic conditions Fe^{2+} salts mostly act as phosphate precipitants after oxidation to the Fe^{3+} form, and the reaction may be written as:

$$Fe^{2+} + ¼\ O_2 + H^+ \longrightarrow Fe^{3+} + ½\ H_2O$$

The optimum pH for phosphate precipitation with ferric iron is the range between pH 4.0 and pH 5.0, while that for ferrous iron is close to pH 8.0 (De Haas *et al.,* 2000). This is likely to be due to the optimum hydroxide formation between pH 6.5 – 9.0 where there was the greatest competition although this was not stated. Standards for discharge of treated effluent to rivers and lakes usually require a pH close to neutral then it is impractical to operate a wastewater treatment process in the optimal range for ferric phosphate precipitation.

2.5.4 Aluminium Salts

Anhydrous aluminium sulphate is also used extensively for phosphate precipitation whilst on the other hand hydrated aluminium sulphate (alum) is commonly used as a coagulant (Morales *et al.,* 1991; De Haas *et al.,* 1991). As with iron, two competing reactions are involved with alum dosing (Wiechers, 1987). The main reactions are the formation of aluminium hydroxides ($Al(OH)_3$), aluminium phosphate ($AlPO_4$) condensation with organic phosphates, and other less stable intermediate reactions.

The equations for the formation of $Al(OH)_3$ and $AlPO_4$ may be summarized as:

$$Al^{3+} + 3H_2O \longrightarrow Al(OH)_3 + 3H^+$$

$$Al^{3+} + PO_4^{3+} \longrightarrow AlPO_4$$

These equations are very simplified because they happen only in perfect conditions (stoichiometric molar ratio of Al:P of 1:1 in $AlPO_4$). The reactions depend on, firstly, pH (for the formation of ion pairs) and secondly precipitation of aluminium-hydroxy-phosphate (Power *et al.*, 1992). In practice, the actual ratio between aluminium dose and P removed varies, similarly as iron, between 2:1 and 3:1 (Wiechers, 1987). According to Power *et al.* (1992), one or more of the hydrolysis products of Al^{3+} (e.g. $Al(OH)_2^+$, $Al_2(OH)_2^{4+}$) are also involved in the precipitation of phosphate. The precipitation reactions are dependent on phosphate concentration and pH, with the optimum pH of 5.5 to 6.5, depending on the composition of the wastewater (Wiechers, 1987).

The formation of aluminium phosphate is thermodynamically and kinetically favoured over hydroxide formation (Jenkins *et al.,* 1971), but to account for the variability, in molar ratio a general formula is often used to describe the precipitate $Al(PO_4)_x(OH)_{3-x}$ (Jiang and Graham, 1998, quoted by De Haas *et al.,* 2000).

2.5.4.1 Poly-aluminium chloride

Poly-aluminium chloride (PAC), sometimes called poly-aluminium hydroxy-chloride, has also been tested for phosphate removal in wastewater treatment. According to De Haas *et al.* (2000), aluminium polymers are easier to use for small processes than pre-polymerised iron species. The advantage of polymers such as PAC is also that they are more efficient in destabilising colloids and less dependent on mixing intensity compared to non-hydrolysed metal salts such as iron sulphate and alum. In practice, when unhydrolysed metal salts are dosed in water, they need reaction time inside the system to form hydroxides and to work as coagulants for the PO_4 and start the precipitation processes.

2.5.5 Precipitation characteristics

The flocs resulting from aluminium salts are less dense and slower to form than those from iron salts. Iron salts directly lead to the formation of well-developed flocs with good settling characteristics but increased sludge production. Aluminium compounds also show a higher efficiency in the neutralisation of surface charges and reduced re-suspension leading to a better coagulation-flocculation process (e.g. removal of turbidity) (D'Elia & Isolati, 1992)

D'Elia & Isolati (1992) also studied PAC in combination with ferric chloride for simultaneous phosphate precipitation in a seasonally overloaded conventional activated sludge plant. Ferric chloride (50mg/L) dosed to the influent of an aeration basin was combined with PAC (30mg/L) in the return sludge, gave a phosphorus reduction of approximately 85%, compared to a range of 50% to 70% phosphorus reduction for 120mg/L ferric chloride only in a previous year for the same plant. This suggests that the PAC + $FeCl_3$ system was more efficient in simultaneous phosphate precipitation.

Iron salt pricing is highly regional and dependent on the local sources and demand for the products. Since the iron salts are mostly obtained from the steel industries, price in UK tends to increase, because of the outsourcing steel production, and is likely to be more expensive in future. The price of ferric chloride in 2008 cost around £65/tonne, in 2013 the price rose up to £125 (price found on alibaba.com accessed in October 2013). This means that for a 45% active ingredient it would be equivalent to £195/day per 100,000 people. Recently, newer sources have been manufactured from scrap materials and acid in order to meet regional demand.

2.5.6 Calcium salts

Lime use was common for coagulation but has been reduced in favour of alternative iron and aluminium coagulants in wastewater treatment recently and over time. Calcium chloride has not been used recently for domestic wastewater treatment or to promote P removal because of its poor solubility and effect on pH. Lime has previously been the third most common chemical used for phosphorus removal (EPA 1976) because of its low cost compared to alum or iron salts, and easier dewatering sludge (Hruschka, 1980), it now plays a minor role because of the necessity to reach a pH above 9.5 to be effective (pH adjustment is done in combination with ferrous sulphate or in side-stream technology), and a major concern is for the additional sludge that is produced (Tchobanoglous *et al.,* 2003).

The major problem with calcium salts is that precipitation needs high pH (around 9), which would be outside of the optimal pH range for most biological processes if calcium is used in a wastewater treatment plant, (De Haas *et al.,* 2000).

Relatively high doses of lime are required to achieve the necessary pH for calcium phosphate precipitation and according to Aspegren (1995), quoted by De Haas *et al.* (2000), at pH 8.6 redissolution of calcium phosphate precipitate occurs to release phosphate concentrations of < 3 mg P/L, while at pH 7, redissolution occurs at concentration around 90 mg P/L.

Problems also come from the maintenance of pipes which experience blockage and accumulation of calcium on the surface of devices such as centrifuges and pumps (Barat *et al.,* 2011). Operational adding of lime is another issue because the solubility in water at 20^{o}C is 1.65g/L which is 220 times lower than alum and 550 times lower than ferric chloride.

Simultaneous P precipitation in activated sludge system using calcium compounds such as lime has been proposed (Jenkins *et al.,* 1971). Phosphate precipitation with calcium results in the formation of apatite species (i.e., $CaHPO_4$, $Ca_4H(PO_4)_3$, $Ca_3(PO_4)_2$) or hydroxyapatite ($Ca_5(PO_4)_3OH$).

De Haan (1981) compared the potential recovery of soluble P at 12 sewage sludges when using lime, ferrous chloride or mixture of ferric and aluminium sulphate. The recovery of sludge-borne P varied from between 20% to 100%. De Haan (1981) stated that while lime treatment had no comparable reaction with soluble phosphorus at neutral pH, compared to the iron or alum 100% recovery as appose to removal could more easily be achieved. Fine & Mingelgrin (1996) confirmed that the addition of Al or Fe salts was a better and more appropriate precipitant for wastewater treatment since it resulted in the formation of less available P species, the lime process being susceptible to resolubilisation. Using lime process for precipitation was however better for P recycle and environment since the apatites could be used directly as soil conditioners.

There has been little recent literature found on calcium salt treatment and not in combination with biological P removal for municipal wastewater treatment. Lime can be used in primary treatment for precipitation (Tchobanoglous *et al.,* 2003), in tertiary treatment, or to maintain the pH and help the alkalinity for the biological processes for example nitrification. Wastewaters that are at higher pH (e.g., pharmaceutical industries) and because it is an inexpensive source of calcium ion and pH adjustment, it has been used for treatment of oily wastes such as palm oil (Tong *et al.,* 2013). It has also been used for the removal of difficult inorganic compounds such as fluoride (Zhang *et al.,* 2011). In these special cases then lime can be used more extensively because it results in better phosphorus removal at lower costs (Spellman, 2008).

More papers have been found to help understand the impact on calcium phosphate precipitation on microorganisms, membranes and other submerged devices (Barat *et al.,* 2006, 2008). Literature on usage of lime and calcium salt for P removal are more often encountered in industrial wastewater treatment such as dairy wastewater (Assobei *et al.,* 2004) and these are reviewed in the next Section.

2.5.7 Calcium and phosphorus

2.5.7.1 Introduction

There has been a great deal of research made on the close relationship between calcium and phosphorus. It is important in the medical area since this is to better understand the formation process for bones, teeth, kidney and gallstones. Calcium phosphate is the most studied biomineral and although the chemical and mineralogical characteristics are well understood, there are still some discussion and debate on some structures, particularly in the formation of biological hydroxyapatite (HAP) at the organic-inorganic interface (van der Houwen *et al.*, 2003; Mekemene *et al.,* 2009).

One of the key elements of this thesis included the suggestion that the chelating mechanism of phosphorus can be done using calcium during wastewater treatment, and thus to understand the role of calcium phosphate precipitation and its stabilisation. The interrelationship between calcium and phosphorus was thought likely to play a role in the speciation of phosphate, and literature was assessed further. The wide variety of organic and inorganic compounds contain in domestic wastewaters, influence the precipitation of calcium phosphate. However, presence of hydrogen ion (pH), magnesium, carbonate and fluoride (to a lesser extent for its low concentration in wastewater) appear to be the most significant disruptive elements (Jenkins, 1971; Maurer & Boller, 1999).

2.5.7.2 Nucleation of calcium phosphate

Calcium phosphate formation is a very complex process in WWTP because of the various parameters which are in this study, apart from calcium and phosphate ions, different super-saturation concentrations, competitive and synergistic ion type, and the more subtle pH and free energy conditions inside the micro environments of microbial flocs (Mekmene *et al.,* 2009; Barat *et al.,* 2011; Habraken *et al.,* 2013). It was therefore hypothesised that nucleation rather than coagulation could be encouraged in BPR processes.

The final effluent for example would be expected to be close to saturation and adding small amounts of calcium phosphate crystal seeds could initiate precipitation.

The classical nucleation theory proposes the stochastic and dynamic association of ions in solution which starting from nuclei by forming crystals after reaching a critical size despite the free Gibbs energy (ΔG) barrier. Nucleation is dependent on degree of saturation (a high σ_{ACP}) increasing inter surface energy α and critical particle size Rc. Gentle mixing could therefore, with a seed, create an excess of free energy that will allow contact for P adsorption and so stimulate nucleation.

Moreover, from this theory, the first species to precipitate is not always the thermodynamically most stable form, but the kinetically the most accessible one. Multistage crystallisation has in all studies, ended up with hydroxyapatite (HAP) as the ultimate, most stable calcium phosphate form. Oswald's general crystallisation rule stated that the least thermodynamically stable phase is the first one to form (van der Houwen *et al.,* 2003; Barat *et al.,* 2011). Forms of amorphous calcium phosphate (ACP) are believed to be the precursors to induce and increase the crystallisation and thus the precipitation (Seckler *et al.* 1996a; Barat *et al.* 2011).

Barat *et al.,* (2011) proposed a classification of the aqueous species and the precipitated solid species (see Table 3) usually implicated in calcium phosphate precipitation process in domestic wastewater and ranked calcium phosphate elements by molar ratio value (Table 4).

Table 3: Presentation of species in aqueous and solid phases

Aqueous species							Solid species
H+	H_2CO_3	H_3PO_4	Ca^{2+}	$CaNH_3^{2+}$	$MgHPO_4(aq)$	$KHPO_4^{--}$	Amorphous calcium phosphate
OH^-	PO_4^{3-}	NH_4+	K^+	$Ca(NH_3)_2^{2+}$	$CaHPO_4(aq)$	$MgCO_3(aq)$	(ACP, $Ca_3(PO_4)_2$ x H_2O)
CO_3^{2-}	HPO_4^{2-}	NH_3 (aq)	$MgOH^+$	$MgPO_4$-	$CaPO_4^{--}$	$MgHCO_3^+$	Hydroxyapatite $Ca_5(PO_4)_3OH$)
HCO_3^-	$H_2PO_4^-$	Mg^{2+}	$CaOH^+$	$MgH_2PO_4^+$	CaH_2PO_4+	$CaHCO_3^+$	

Source Barat *et al.,* (2011)

Table 4: Calcium phosphate phases ranked by molar ratio Ca/P

Phase	Reference	Composition	Molar ratio Ca/P
Brushite	DCPD	$CaHPO_4.2H_2O$	1.00
Monetite	DCPA	$CaHPO_4$	1.00
Octocalcium phosphate	OCP	$Ca_4H(PO_4)_3.2.5H_2O$	1.33
Amorphous calcium phosphate	ACP	$Ca_3(PO_4)_2.xH_2O$	1.50
Tricalcium Phosphate	TCP	$Ca_3(PO_4)_2$	1.50
Hydroxyapatite	HAP	$Ca_5(PO_4)_3OH$	1.67

Classification proposed by Barat *et al.,* (2011)

The most common sequence transformation found is: amorphous calcium phosphate (ACP), brushite or dicalcium phosphate dehydrate (DCPD), octacalcium phosphate (OCP) then hydroxyapatite (HAP), the mechanism of the charged solutes aggregation is driven by the gain of entropy combined with the release of hydration water (Marshall *et al.*, 1969; Mekmene *et al.,* 2009; van der Houwen *et al.*, 2003).

A study on biometric precipitation of calcium phosphate was made by Habraken *et al.* (2013) who could were able to reconcile observations of non-classical ACP formation with classical theory. They stated that the ACP formation did not conform to classical nucleation theory, if calcium phosphate nucleation was only a function of saturation.

If the thermodynamic barrier for nucleation was lowered during pre-nucleation complexes and particle size formation, then the interfacial free energies that were in the system changed and the inconsistencies were sufficient to overturn the classical theories.

Nucleation models involving pre-nucleation clusters have been proposed in many studies with complex species formed with their own characteristic calcium solubility (Habraken *et al.,* 2013). Their calculation found that the chemical development indicated a triangular arrangement of phosphate around the calcium ion, and through their experiments Habraken *et al.* (2013) revealed that what was observed as calcium phosphate pre-nucleation clusters was in fact triphosphate ion-association complexes that aggregate around calcium ion.

2.5.7.3 The thermodynamic growth in complex system

The formation of calcium phosphate, their aggregations and their kinetics have been reported based on the observations made on the aqueous species PO_4^{3-}, HPO_4^{2-} and $H_2PO_4^-$ behaviour, and although the results could be reproducible in WWTP this was unlikely. They were generated from synthetic solutions used with controlled concentrations of calcium and phosphate in relatively clean waters.. Table 3 from Barat *et al.* (2011) provides some of the inorganic species that are involved in wastewater. The microbial activity and the level of organic components that are present change the chemistry of the solution. The kinetics and the thermodynamic barriers are then unaccountably complex combinations of processes that occur in clean solutions. Moreover in WWTP there will be pH buffering, changes in temperature and RedOx, thus increasing the complexity of the interactions further. Nevertheless these model systems could be an indication of what would happen in a WWTP. In this research however as well as the multi-RedOx zones of standard P removal plant (anaerobic, anoxic and aerobic), the final effluent after membrane filtration reduces the problem to only soluble species < 0.4 μm and positive RedOx. The level of ΔG then depends on supersaturation of ACP (σ), the interfacial energy of the nucleating phase (α) and the size limit dependence of interfacial energy or the critical radius (*Rc*).

2.5.7.4 Mechanism of aggregation

This research will not attempt to develop models that predict the different species formed as the dynamic system is too complex for the objectives of the present thesis. On the other hand, trying to understand the role of these complex precipitates that occur in the different stages of the physical laboratory scale experiments could aid further work Therefore some potentially important representative phosphorus chemical precipitates are referred to the next Section.

2.5.7.4.1 Nucleation of calcium phosphate dynamic

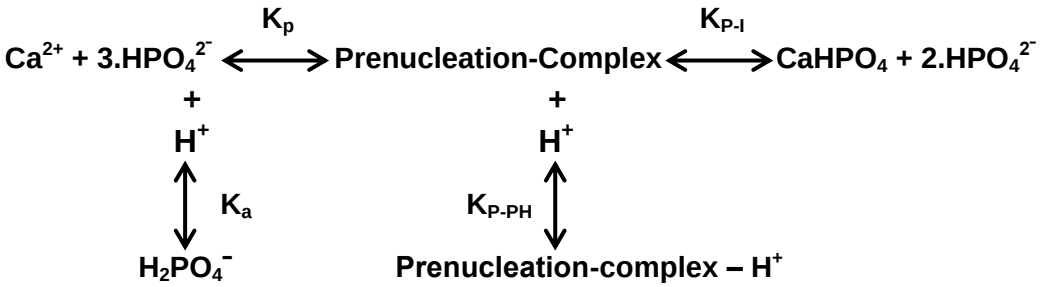
Habraken *et al.,* (2013) described a general model of the pre precipitation reactions :

$$Ca_x(HPO_4)_y(H_2PO_4)_z^{2x-2y-z} \xleftrightarrow{K_{Eq}} x.Ca^{2+} + y.HPO_4^{2-} + z.H_2PO_4^-$$

The process consists of a descriptive equilibrium between pre-nucleation complexes, ion pairs and ions with their corresponding equilibrium constants (K_{Eq}, K_a, K_p, K_{P_PH} and K_{P_I}) (Habraken *et al.,* 2013):

Two processes occur:

1. Initial calcium triphosphate complexes form (pre-nucleation);
2. Three-dimensional branched polymeric structures of amorphous calcium triphosphate (ACP) (pre-nucleation) are formed;



These basic combinations evolve into polymeric assemblies (Habraken *et al.*2013). However the process is limited by reaction time as it involves hydrogen bonding, to form the post-nucleation clusters. Chemically [Ca^{2+}] is gained and 2[H^+] are lost. These post aggregations start to precipitate as amorphous spheres, the first crystal structure is formed from octocalcium phosphate which is calcium deficient (OCP-Ca def) ($[Ca_6 (HPO_4)_4 (PO_4)_2]^{2-}$ (Ca/P = 1.0)), this structure then ages with more calcium ($Ca_8 (HPO_4)_2 (PO_4)_4$ (Ca/P = 1.33) to finally evolve into apatite (AP). Basically, all are fractals of $Ca_2 (HPO_4)_3^{2-}$.

2.5.7.4.2 Calcium phosphate precipitation in activated sludge

Carlsson *et al.,* (1997) investigated the conditions for calcium phosphate to precipitate during EBPR. Their experimentswere in a hard wastewater area with 100 mg/L of Ca and 15 mg/L of Mg (from Sjolunda WWTW, Sweden), they increased artificially the phosphorus content (with sodium phosphate) and the COD with acetate (i.e., sodium acetate), and did the experiments at 14°C using beakers.

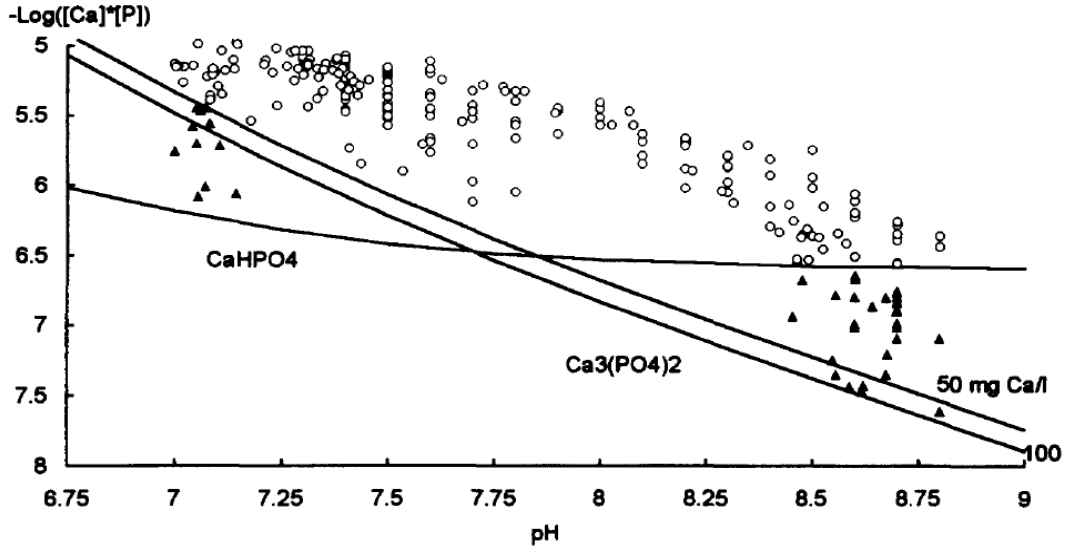
They concluded that in most municipal hard wastewaters, the precipitation of calcium phosphate was not a significant P removal mechanism but, some of Carlsson's results can be interpreted differently for this research:

1. The Ca/P ratio for precipitation was around 1–1.3;
2. The equilibrium equations could not predict the conditions for calcium phosphate precipitation to occur or the extent to which it occurs;
3. The precipitation of calcium phosphate species were pH dependent and thus at neutral pH $CaHPO_4$ is formed, and at pH ~8.5 $Ca_3 (PO_4)_2$ is formed. Consequently,

these species determine the saturation concentration below which P or Ca specific concentrations will encourage redisolution (see Figure 3);

4. For precipitation at neutral pH, P should be at least 50 mg/L when Ca is minimum 100 mg/L, and higher pH if the Ca is below 50 mg/L. The phosphorus concentration needed for precipitation was reduced as the pH increased.
5. Barat *et al.* (2011) stated that in hard wastewaters, biologically induced precipitation could contribute significantly to the amount of P removal and was frequently reported in the literature.

Figure 3: EBPR profile measurements of calcium and phosphorus molar ratio (Carlsson *et al.*, 1997)



○: calcium; ▲: phosphorus. The Lines represent the theoretical conditional solubility products for $Ca_3(PO_4)_2$ and $CaHPO_4$ at 25°C

The experiments made by Barat *et al.*, (2011) proposed a model which reproduced the dynamics of amorphous calcium phosphate precipitation then the crystallisation into hydroxyapatite. Barat et al proposed the model could characterise the Enhanced Biological Phosphorus Removal (EBPR) process but they did not comment on EBPR as a combination of biological, physical and chemical processes.

There were well known biological processes models for wastewater treatment notably the International Water Association (IWA)(ASM2d) which includes the precipitation processes (BioWin). Barat et al criticised this and other models because they ignore the possibility that calcium phosphate redissolves as the pH decreases. Barat *et al.* (2011) suggested that their model was a development and did overcome the above problems.

Their conclusions from the validation of the model were as follow:

1. Ca^{2+} was not involved in the biological phosphate dynamics and thus was not affecting the PAO metabolism, unlike potassium or magnesium (Barat *et al.,* 2005), they implied that calcium ion difference (ΔCa^{2+}) removal was mainly due to precipitation;
2. Ca^{2+} remained constant in the anaerobic phase even with increasing PO_4-P concentration, only during the aerobic conditions was there a significant decrease, where it was related to a pH increase (CO_2 stripping) and high PO_4-P level;
3. Below a certain level of PO_4-P due to biological uptake, the concentration of calcium increased since additional phosphate was taken up by the phosphorus accumulating organisms (PAOs). The release of calcium came from ACP dissolution mainly caused by these low PO_4-P concentrations that changed the equilibrium conditions.
4. Calcium carbonate formation and precipitation occurred during the aerobic phase however not as calcite crystals despite the appropriate thermodynamic conditions. Calcite formation did not occur due to the inhibiting properties of PO_4-P on the calcite formation.
5. Thermodynamically ACP was formed in the anaerobic phase (not as precipitate due to low pH) and continued to form in the aerobic phase and partially dissolved due to an unbalance in the equilibrium with PO_4-P at low P concentrations.

A review of the Barat *et al.* (2011) model concluded that it was able to reproduce the calcium phosphate precipitation processes under different conditions (no precipitation / little precipitation / precipitation-dissolution). Nevertheless, the biological PO_4-P removal was difficult to predict with the model when there were pH variations, but it was also affected by P/COD ratio, PAO/GAO ratio, and PAO metabolic pathway (literature review section 2.6.9.2).

The Barat et al. model for the metal cations concentrations helped to understand ion dynamics in the EBPR, and were expected to be a good indication of what would occur in our laboratory experiments but not help to understand the impact of COD/P or nature of the carbon on EBPR. The chemistry of calcium phosphate in milk, the proposed additional carbon, was therefore reviewed in the next Section

2.5.7.4.3 Calcium phosphate complexes in milk

Van Kemenade & de Bruyn (1987) mentioned that the precursor amorphous form before crystallisation of HAP does not occur in milk because of the protein sequestration by caseins. In a study made by Pouliot *et al.* (1991) consisting of inducing calcium phosphate precipitation from whey permeate. The study could achieve this by seeding with crystals of dicalcium phosphate as nuclei and this was proposed for our experiments. More extensive crystallisation followed that led to a reduction of both calcium and phosphate. Pouliot *et al.,* (1991) used the dicalcium phosphate dihydrate (DCPD) to achieve complete calcium phosphate precipitation but at 50°C, and pH 8.0. The precipitate had Ca/P = 1.33 ratio.

The research generated other relevant conclusions (Pouliot *et al.,* 1991):

1. At 20°C the precipitate had a Ca/P = 1 ratio and if pH was above 6.6, then there was no decisive influence on nature and amount of precipitate.
2. A pH between 6.5 and 8.0 was favourable to form calcium phosphate;
3. The adsorption of calcium phosphate can be created on existing solids particles reducing the disrupting effect from inhibiting reagents;
4. This was explained thermodynamically by an increase in free Gibbs energy (ΔG) that induced nucleation to the solid particles (see also Section 2.5.7.2).

Mekemene *et al.,* (2009) used other minerals to precipitate calcium phosphate and adjust the pH in milk. The Ca/P ratio did not influence the precipitation at acid pH 4.6 - 6.0. but above this level from pH 7.0, the Ca/P ratio was decisive with an optimal ratio of Ca/P = 1.50 (at pH 7.0 precisely).
At neutral pH and above, they concluded:

- Ca/P ratio ≤ 1, the calcium concentration was the limiting factor;
- Ca/P ratio ≥ 2, the phosphate concentration was the limiting factor.

2.5.7.4.4 Effect of organics on calcium phosphate formation

As it is standard practice to artificially increase the COD level with an exogenous carbon source to stimulate growth of PAOs, it was important to understand if the calcium phosphate formation could be either affected in quality or in the precipitate by organic materials. Habraken *et al.,* (2013) for example noted pure amorphous calcium phosphate was necessary to produce crystals and also described the fractal stages involved in the formation of Ca_2 $(HPO_4)_3^{2-}$. Impurities and organics were also reported to reduce PO_4P removal through precipitation because of increased solubility (Barat *et al.,* 2011).

van der Houwen *et al.,* (2003) studied the influence of acetate and citrate ligands, on calcium phosphate precipitation and its quality. Hydroxy-apatite (HAP) precipitation was affected by carboxyl groups especially citrate. The problem during the crystal formation was actually mainly due to the protein extremities attached to the carboxyl groups that interacted with $(OH)^-$ and /or $(PO4)^{3-}$/$(HPO_4)^{2-}$ nuclei. Gaucheron *et al.,* (1996) also investigated the impact of organic acids present in milk on precipitation. They measured the effect of citrate at 1500-2200 mg/kg compared to chloride at 1000 – 1100 mg/kg.. Gaucheron *et al.,* (1996) also investigated acetate and concluded unlike citrate and salt it did not interfere with the calcium phosphate formation. Acetate is expected to be in the greatest quantity after lactic acid during the decomposition of fatty acids in milk

Sodium chloride was used by Gaucheron *et al.,* (1996) to balance the ionic strength it resulted in both sodium and chloride being present in the imperfect HAP. The calcium phosphate precipitate molar ratios were all changed giving a non-stoichiometric ratio precipitant normally the HAP calcium phosphate ratio is 1.67. It was therefore concluded that these impurities will take part in the binding process between calcium and P, and interfere with the quality of the recovered P but that these competitive reactions should not be enough to prevent Ca-P precipitation.

2.5.7.5 Calcium chloride

Several investigations have used $CaCl_2$ in milk research and industrial applications to supplement calcium in milk or cheese or to restore the calcium balance (Williams *et al.*, (2005); Hallen *et al.,* (2010). It has also been used to investigate the interactions between calcium and other biological environment for example (Carlson *et al.,* 1960; De Kort *et al.,* 2009).

It is a readily quantified source of free [Ca^{2+}] in solution De Kort *et al.,* (2009) for example used it to assess the calcium binding capacity of orthophosphate and polyphosphate in both forms as organic and inorganic. This study was relevant to domestic wastewater treatments where removing soluble phosphorus is the most important and which originates from both organic and inorganic sources.

Therefore in this research calcium chloride ($CaCl_2$) was chosen as an adjunct because of this previous work and its solubility without increasing pH as happens with lime.

2.5.8 Biological treatment

2.5.8.1 Introduction

In activated sludge, enhanced biological phosphorus removal (EBPR) can be easily implemented and is widely accepted to be one of the most economical and environmentally sustainable options for P-removal (Oehmen *et al.,* 2007; Broughton *et al.,* 2008). Recirculating the sludge anaerobically with soluble substrates contained in the influent, namely volatile fatty acids (VFAs) causes the mobilisation of P (de Lucas *et al.,* 2007a,b) which is then taken up again and with additional P when aerobic conditions are restored. The ordinary heterotrophic bacteria in ASP are very effective at fermenting (anoxic/anaerobic) complex organic substrates contained in wastewaters into simple soluble VFA (Mino *et al.,* 1998, de Lucas *et al.,* 2005a, 2007a, b). About 95% of the total bacteria contained in activated sludge can carry out this metabolic step (de Lucas *et al.,* 2007b).

The necessity to increase the carbon ratio to remove the nutrient (C/P or C/N) has been widely reported by Liu *et al.*, (1997); Schuler and Jenkins, (2003); Ge *et al.,* (2010) especially for weak influent.

Compared to chemical P removal the advantages are (Metcalf & Eddy, 2003):

- Limited sludge production: no extra management or disposal costs;

- Potential P-recovery: ecological spreading on culture fields;

- Limiting side effects on environment when releasing the final effluent.

2.5.8.2 Microbial stage processes:

There are a number of commercial variants in the basic combination of stages used in EBPR processes and these are reviewed in Metcalf and Eddy (2003) for example. All the processes follow the standard redox sequence shown in Figure 4

Anaerobic stage:

Heterotrophic bacteria, in the absence of suitable electron acceptors, use VFA from the influent to store potential energy within the cells as poly-hydroxyalkanoates (PHA), poly-hydroxybutyrate (PHB), poly-hydroxyvalerate (PHV) or poly-hydroxy-2-methylvalerate (PH_2MV) depending on the nature of VFA provided (Smolders *et al.,* 1994a; Oehmen *et al.,* 2005c). PHA are mobilised during time of starvation or during absence of suitable exogenous carbon sources (Ushino *et al.,* 2007).

The hydrolysis of polyphosphate (poly-P) and glycogen provide internal energy to form PHA in the PAOs who consequently release P into the liquid phase as orthophosphate (PO_4^{3-}-P) (Mino *et al.,* 1998; Seviour *et al.,* 2003).

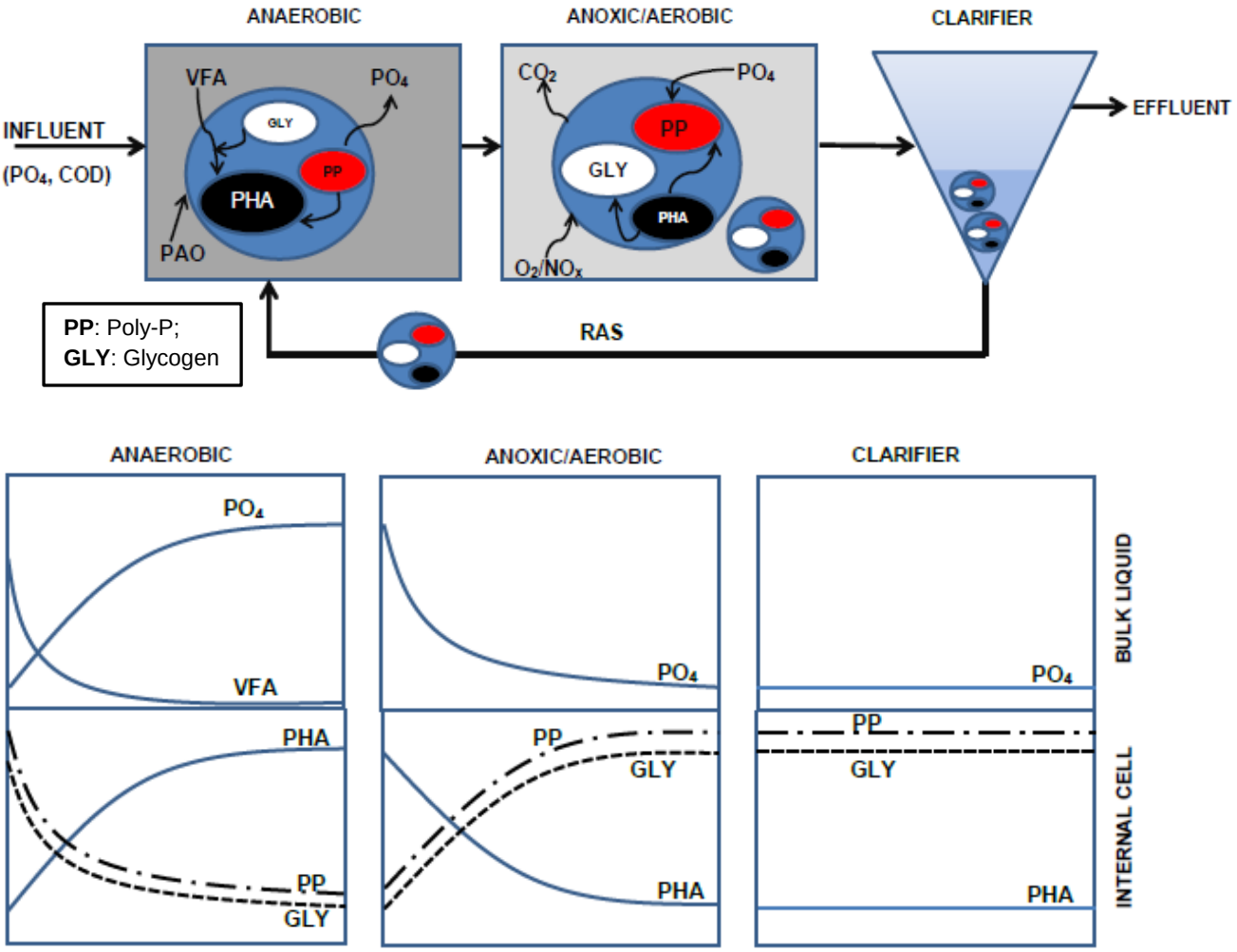
Anoxic/Aerobic stage:

In these phases, heterotrophic bacteria use the stored PHA formed during the anaerobic phase as energy source, some bacteria use nitrate as the electron acceptor during the anoxic phase to take up orthophosphates, and other bacteria during the aerobic phase use oxygen as the electron acceptor to take up the orthophosphate, and to replenish the glycogen pool in their cells. These combined processes will take up more ortho-P from the liquid bulk than released during the anaerobic stage. The PAO enriched biomass continues to grow successfully despite these changes in environmental conditions (Smolders *et al.,* 1995; Mino *et al.,* 1998). Nitrite has been recognized as an inhibitor in microbial metabolism. The NO_2-N inhibits the anoxic and aerobic P uptake, leading to deterioration of biological P removal (Zeng *et al.,* 2011).

After aerobic stage:

The net P-removal is achieved by taking out the excess sludge as the phosphorus is sequestered in the activated sludge. Figure 4 describes the EBPR PAOs, P mass balance.

Figure 4: Conceptual PAO activity during EBPR process



2.5.8.3 Main microbial population involved in EBPR: PAO.

Using the fluorescence in situ hybridization (FISH) (Seviour *et al.,* 2003), it was observed by Hesselman *et al.* (1999) that a species named "*Candidatus Accumulibacter Phosphatis*" known as *Accumulibacter* were dominant in EBPR with efficient P removal because they were able to perform the anaerobic P-release, by breaking down poly-P into ortho-P, followed by the anoxic/aerobic P-uptake cycle using PHA (Hesselman *et al.,* 1999; Oehmen *et al.,* 2007a, b). Other studies suggest that there are other groups of PAOs (Wong *et al.,* 2005) although it has not been possible to isolate a group of PAO (Oehmen *et al.,* 2007a, b). Uncertainties remain therefore concerning their taxonomic status.

2.5.8.4 Main microbial population competing with PAOs: GAO.

Glycogen-accumulating organisms (GAO) were first discovered in 1977 and their involvement in the EBPR was suggested by Cech & Hartman (1990) who observed large numbers of GAO-bacteria in a plant fed with glucose which was showing poor P removal (Seviour *et al.,* 2003).

From an EBPR point of view they are referred to "*Candidatus Competicibacter Phosphatis*" *Competicibacter, added* because firstly they compete very efficiently against PAOs for the available substrates and secondly GAOs do not release or accumulate P, and reduce EBPR efficiency. GAO store glycogen aerobically, and ferment their stored glycogen anaerobically to generate energy, rather than take up VFA to store them as PHA (Mino *et al.,* 1998).

2.5.8.5 Factors influencing PAO-GAO competition.

Other studies have contributed to a better understanding of the factors influencing the microbial competition and optimisation of EBPR process. The main factors studied are summarised in the Table 5 below:

Table 5: Main factors studied in laboratory for GAO-PAO competition.

Influent carbon source (HAc, HPr)	Oehmen *et al.*, (2005b,c, 2006a,b).
Influent phosphorus to carbon ratio	Liu *et al.*, (1997); Schuler & Jenkins, (2003).
pH	Filipe *et al.*, (2001b); Schuler & Jenkins, (2002).
Temperature effects	Whang & Park, (2006).

There is however general concerns in the literature about competition between PAOs and GAOs:

- At higher wastewater temperature (higher than 20 °C), GAOs are favoured (Whang & Park, 2006);
- The smallest short chain fatty acid (SCFA) HAc and HPr are the most commonly used in experiments since they are dominant in full scale treatment plants (Mino *et al.*, 1998). GAOs are not able to take up HAc and HPr as efficiently as PAO.

  Zheng *et al.* (2006) observed that a higher HAc to HPr ratio can favour PAO to GAO and give a more stable and reliable EBPR process then when using either HAc or HPr as sole carbon This is because the use of one exclusive SCFA can favour the GAO strains occur that are capable of taking up at the same rate as the PAOs with specific SCFA (Oehmen *et al.,* 2005b, 2005c, 2006a).

- P/VFA ratio (above 0.12 P-mol/C-mol) is reported as an advantage to PAOs activity. A lower ratio (lower than 0.02 P-mol/C-mol) creates a P deficit which inhibits the PAOs growth, and thus is beneficial to GAOs (Liu *et al.,* 1997; Schuler & Jenkins, 2003)
- Many studies such as Felipe *et al,* (2001b, c), Schuler & Jenkins, (2002), Oehmen *et al.,* (2005a) stated that a pH higher than 7.25 is necessary to have an efficient EBPR process. This is explained by the potential energy needed, in the anaerobic conditions to break down poly-P (creating more energy) whilst maintaining pH for the trans-membrane uptake of HAc (Smolders *et al.,* 1994a, Filipe *et al.,* 2001d).

  It is thought that GAOs lose their competitive advantage over PAOs since the latter create more energy from poly-P break down with glycogen compared to the GAOs using anaerobic metabolic steps and consequently can less rapidly absorb HAc (Filipe *et al.,* 2001a). Non-VFA carbons are also used to promote PAOs and this will be discussed in detail in the next paragraph.

2.5.8.6 EBPR process failure and performance deterioration.

Efficient biological treatment of domestic wastewater to remove P, especially when its raw influent is weak due to rainfall, is difficult to achieve. Although wide spread, the EBPR process is often destabilised by alternating dilute wastewater but also by other different factors. This presents an undesirable level of unreliability when trying to meet the international standards and requires further research.. These process upsets can be divided into either environmental and/or operating issues (Seviour *et al.,* 2003; Oehmen *et al.,* 2007).

- The most common cause is a reduction in sewage strength as a result of heavy rainfalls, as often encountered in UK, that have the potential to reduce the C/P to below the critical ratio by diluting the available carbon source.
- During the denitrification (or pre-denitrification), if nitrite or nitrate (NO_2^--N and NO_3^--N) enters the anaerobic tank this will inhibit PAOs P release activity and so reduce the EBPR process Saito *et al*. (2004).
- The presence of NO_2-N and NO_3-N with substrates automatically induces anoxic conditions that lead to a rapid preference for denitrification over phosphorus release because of the faster heterotroph uptake and growth rate compared to anaerobic P metabolism. This heterotrophic growth leads to consumption of available VFA during de-nitrification provoking a decrease in EBPR efficiency (Kuba *et al*., 1994; Puig *et al*., 2007);

- Jansen *et al.* (2002) reported that upset disruptions to the normal operation of WWTW are often caused by the lack of maintenance but also by lack of understanding by the operators;
- A predominance of GAOs, which compete for VFAs with PAOs, has been hypothesised to be the main cause of the deterioration of the EBPR process performance (Liu *et al*., 1997; Filipe *et al*., 2001a; Oehmen *et al*., 2007).

2.5.8.7 Other microbial population.

Most bacteria found in the wastewater can reduce COD into easily assimilated substrates but are often referred as the ordinary heterotrophs organisms (OHO) to differentiate them from PAO. Thus as noted, the status of the PAO as subgroup of heterotrophs remains unclear. Autotrophic bacteria, cyanobacteria, protozoa, rotifers, nematodes and a few others invertebrates can also reduce the COD, converting it into more available substrates (de Lucas *et al.,* 2005a).

Many studies are trying to identify Denitrifying Phosphorus Accumulating Organisms (DPAO) in activated sludge system (Kuba *et al.,* 1996). DPAOs could be capable of denitrifying and removing phosphorus simultaneously (Kuba *et al*., 1996). The advantages were summarised by Zeng *et al.,* 2011:

1. Energy saving by reducing aeration;
2. Reduction of supplementary carbon;
3. Reduction of sludge production.

Nevertheless, the existence of the DPAOs is not certain and their activity inconsistent. Kuba *et al.* (1996), and Hu *et al.* (2002) the major protagonists reported DPAOs activities in anoxic conditions from 15 to 100 % of the aerobic P-removal activity.

Hu *et al.* (2002) published several studies where DPAOs were less efficient at uptake P (about 20% - 30%) compared with the oxygen using PAOs (APAOs). Smolders *et al.* (1994) and Kuba *et al.* (1996) have analysed the energy balances where δ_o and δ_n represent the amount of adenosine triphosphate (ATP) produced from oxygen or the nitrate as electron acceptors respectively to oxidize 1 mole of $NADH_2$ (electron transport for phosphorylation).

Smolders *et al.* (1994) and Kuba *et al.* (1996) proposed two alternative reactions:

Aerobic condition: $NADH_2 + \frac{1}{2} O_2 \rightarrow \delta_o ATP + H_2O$ (1)

Anoxic condition: $NADH_2 + \frac{2}{5} HNO_3 \rightarrow \frac{1}{5} N_2 + \delta_n ATP + \frac{6}{5} H_2O$ (2)

Energetically the δ_n of DPAOs calculated by Kuba *et al.* (1996) was on average equal to 1.0 mole ATP per mole of $NADH_2$ and δ_o of APAOs proposed by Smolders *et al.* (1994) was about 1.85 mole ATP $mole^{-1}$ $NADH_2$.

Garcia-Usach *et al.* (2010) using (1) and (2) developed kinetics to the stoichiometric biochemical pathway relationship between $Y_{PHA, O2}$ and $Y_{PHA, NO}$ using aerobic parameters:

$$Y_{PHA, NO} = \frac{5.6}{8.65}\ 2.86\ Y_{PHA, O2} \quad (3)$$

Similarly the anoxic growth of heterotrophs can be found using:

$$Y_{H, NO} = [1 + (1 - Y_{H,O2}/ Y_{H,O2}) .\ \frac{5.6}{8.65}\ 2.86]^{-1} \quad (4)$$

More details can be found for (3) and (4) in Garcia-Usach *et al.* (2010) but the conclusion was that DPAO denitrification, at maximum yield of P-uptake by DPAOs, was 30% of the energy yield of the heterotrophic denitrifying bacteria OHOs (Hu *et al.*, 2002). It was not thought necessary to incorporate these reactions into the standard IWA model (Activated Sludge Models (ASM) (Henze *et al.,* 2002). The standard model which includes existing routines for P removal is described in Figure 7.

ASM1: model measures the component concentrations in sludge and wastewater;

ASM2: model includes ASM 1 with P removal;

ASM2 (d): model includes ASM2 with simultaneous nitrification-denitrification;

ASM3: ASM2 (d) + correct some defects of ASM, predicts oxygen consumption, sludge production and storage of organic substrates.

Modelling has been used very successfully to predict the potential for settling problems in l wastewater treatment using activated sludge and this is reviewed in the next Section.

2.5.8.8 Microbial Morphology/Settlement and EBPR

There are aspects of morphology that could affect EBPR. Modelling described in the previous Section has indicated that in general bacteria which are filamentous in shape are better adapted to reactors with low easily assimilible substrate concentrations typical of nutrient removal WWTP. In contrast at higher concentration and particulate substrates favour flocculent morphology. Thus the filamentous bacteria are likely to be significant competitors for the VFA needed by PAO. Filamentous are most notorious for causing activated sludge settlement problems and the separation of the liquid phase from the MLSS becomes complex as it was noted originally by Eikelboom.

Most scale down model activated sludge plants also suffer from poor settlement or bulking and this influenced the use of membranes in this project discussed in the following Section.

The sulphur bacteria are the most commonly found filaments with the most prolific being, for example, the *Thiothrix* and Eikelboom type 021N (Vaiopoulou *et al.,* 2007), *Sphaerotilus natans, Haliscomenobacter hydrossis* (Gaval & Pernelle, 2003). See Figure 5 for an example of the Eikelboom Type 021N groups.

Figure 5: Classification of Eikelboom type 021N bacteria strains

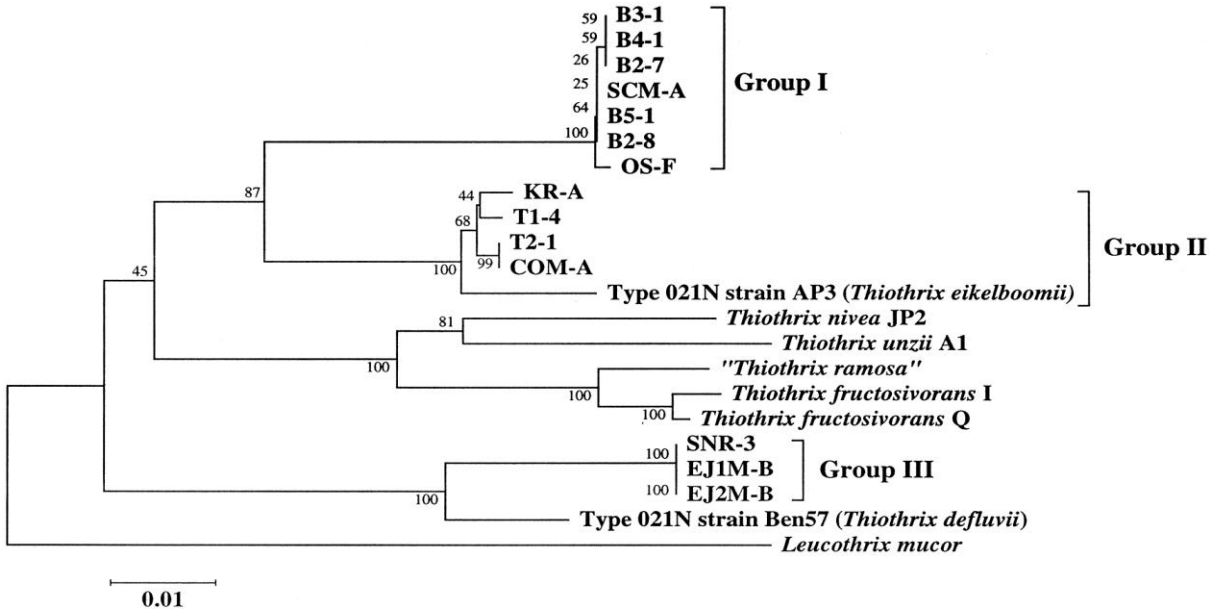


Figure 5 Phylogenetic tree derived from 16S rDNA sequences showing the position of the Eikelboom type 021N strains. The tree was calculated using the neighbour-joining method. The bar represents 1% estimated sequence divergence. Numbers at nodes represent percentage bootstrap values. The GenBank accession numbers of the reference strains used in the phylogenetic analysis are as follows: *T. defluvii*, AF127020; *T. fructosivorans* I, L79963; *T. fructosivorans* Q, L79962; *T. unzii*, L79961; *T. ramosa*, U32940; *T. nivea* JP2, L40993; *Leucothrix mucor*, X87277. From KANAGAWA et al., (2000)

General operational problems from excessive growth of filamentous bacteria (Kanagawa *et al.,* 2000; Vaiopoulou *et al.,* 2007) are :

- Poor settlement of activated flocs (bulking);
- Prefer readily biodegradable organic substrate (competing with PAOs);
- Take up rapidly VFA when they are low (worsen the situation);

Main causes (WWTW stresses) identified for dominant filamentous growth were summarized by (Gaval & Pernelle, 2003):

- Oxygen deficiencies (most common problem);
- Nutrient deficiencies;
- Decrease in food / micro-organisms ratio (F/M);

- Presence of reduced sulphur compounds;
- Wastewater composition, (e.g. sulphur and or anaerobic sewage).

The literature suggests that these bacteria thrive most commonly either in the aerated or the anoxic zone. They could be found in the anaerobic tank because of the recycling into the tank, where the anaerobic conditions generates sulphide (providing advantage over floc-forming bacteria).

. Flocculent bacteria generate exocellular polymeric material (EPM) they are grouped in the genus zoogloea (animal glue), although this is likely to revised as genomic analysis is applied. Their high water containing gels can cause viscous, poorly settling and compacting sludge with problems for dewatering. In common with the filaments they can also use nitrate for respiration (Montoya *et al.,* 2008). According to Miqueleto *et al.* (2010), different factors influence the composition, structure and quantity of the extracellular polymeric substance (EPS), such as found in zoogloea. These include for example inorganic species (P and Ca concentrations), or the nature of substrate fermentation conditions. The EPS matrix is strongly adsorbent and will aggregate bacterial cells in flocks and biofilms with water retention. They are capable of digesting exogenous organic macromolecules with their enzymes for the accumulation of nutrients from the environment. The high production of insoluble EPS observed in wastewater treatments does have disadvantages causing bed clogging in fixed-bed systems, or problems with biomass flocculation (or granulation). One of the new techniques which can prevent most of these morphology problems is the use of membrane bioreactor technology discussed in the next Section.

2.5.9 Membrane bioreactor

2.5.9.1 Background and history

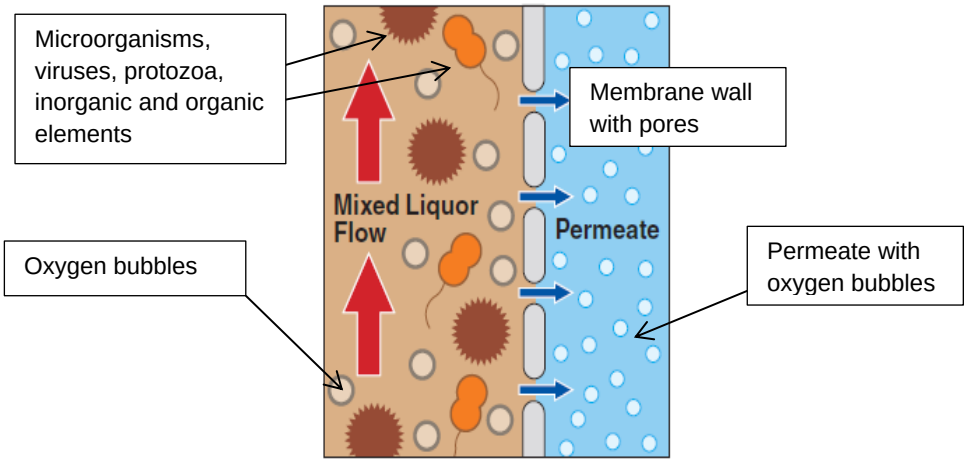
In general, the possibilities and capabilities of the conventional treatments, activated sludge, biofillers and rotating biological contactors (RBC) are widely known (Tchobanoglous et al 2003) thus it will not be detailed in the literature review.

Using a membrane as a tool for separation seems to date from the 18th century as in 1748 L'Abbe Nolet used the word osmosis to describe the permeation water through a diaphragm made of pig's bladder (Baker, 2004). The commercialisation apparently started in 1930 to produce pure drinking water (Pouliot, 2008). In 1960, students Sidney Loeb and Srinivasa Sourirajan at the University of California in Los-Angeles (UCLA) worked on a water desalination program and developed high-flux anisotropic reverse osmosis (RO).

The effective way to produce RO membranes and the discovery of asymmetric membranes is viewed as the cornerstone of industrial membrane processing, and probably the starting point of modern membrane science (Pouliot, 2008).

Membrane Bioreactors (MBRs) are typically used in a multistage treatment scheme and may be either anaerobic (AnMBRs) or aerobic (AeMBRs) for liquid /solid separation through a porous membrane acting as a barrier to most particles present in the mixture with a diameter bigger than the pore size as described in Figure 6 (Guglielmi *et al.,* 2007; Evren Ersahin *et al.,* 2012).

Figure 6: Physical filtration for particulate process.



The top three commercial suppliers are Kubota®, Mitsubishi Rayon and Zenon (now G.E.). In 2009 they were present in 200 countries with 4400 installations. At present in the UK there are around 6500 sewage works but only 3 MBR (Smith *et al.,* 2012).

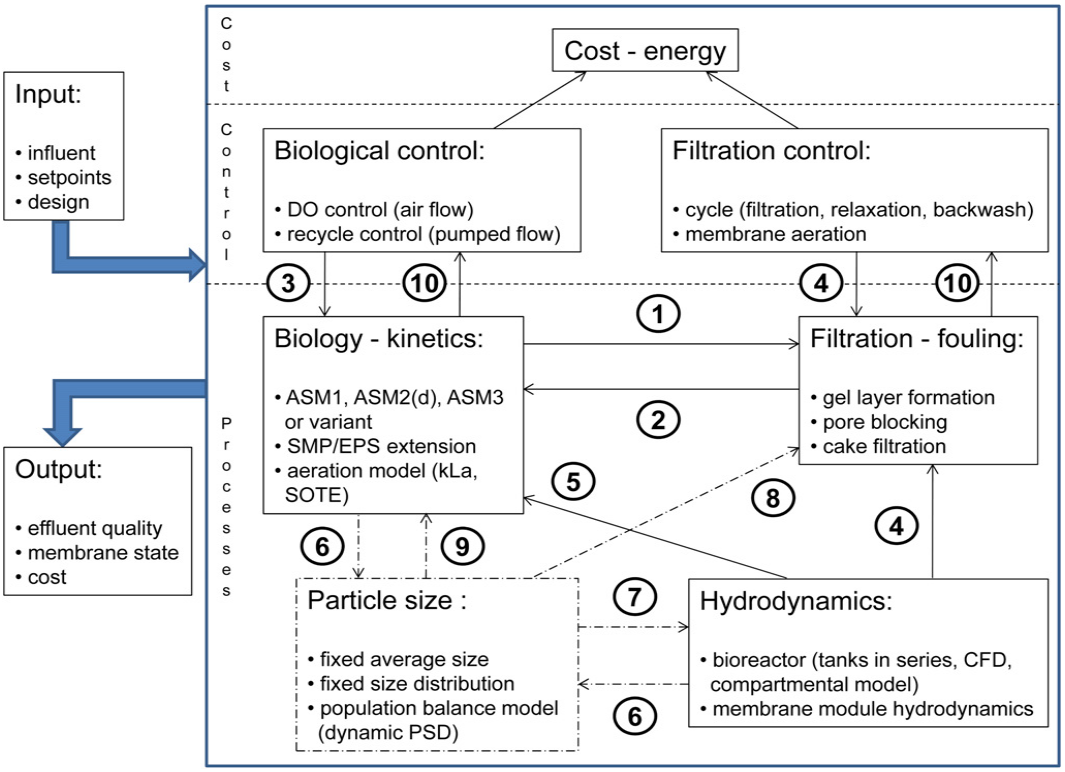
There are also what are referred to as dynamic membranes (DM), also called secondary membranes, which has the filtering process actually formed by microbial cells and flocs on an under-laying support material such as membrane or filter cloth. In this configuration, a biomass layer made of organic and colloidal particles are formed on the support material and will act as a filtration layer that potentially prevents fouling and are similar in mechanism to biological or trickling filters (Evren Ersahin *et al.,* 2012).

As it was explained earlier, the increasing demand for high quality for drinking water or reuse in the future, might push membrane bioreactor (MBR) technology to play an increasing role that has not yet reached its full development potential (Le-Clech, 2010).

This research and literature will be focused on AeMBRs,.the main reason was when compared to conventional aerobic treatment using gravity sedimentation, superior effluent quality, easier operation smaller size (Smith *et al.,* 2012) and much less pre- and post-treatments (Alvarez-Vazquez *et al.,* 2004).

The overall system is shown in Figure 7 from Naessens *et al. (*2012). Table 6 presents the different existing membranes uses and their general specifications.

Figure 7:Illustration of the complex interactions between different processes in an MBR and interaction with the control layer and costs



SMP/EPS: Soluble Microbial Products (formation/degradation) / Extracellular Polymeric Substance;

kLa: oxygen mass transfer rate construction;

SOTE: parameters adjusted to achieve a Standard Oxygen Transfer Efficiency;

PSO: Particle Size Distribution (in suspension)

CFD: Computational Fluid Dynamic which provides a method for prediction.

Example: predicts effects on hydrodynamic according to mixing energy.

Table 6: Membranes specifications

Parameter	Tubular High Rate	Tubular Membrane	Hollow Fiber	Flat Plate
Estimated Life	10+ years	10+ years	5-7 years	10+ years
Pore size[(a) (*)]	MF, UF, NF	MF, UF, NF	UF, NF	UF, NF
Membrane materials [(a)(b)]	Inorganic, polymeric	Inorganic, polymeric	Inorganic, polymeric	Inorganic, polymeric
MLSS Range (mg/L)	1000 - 20,000	1000 - 20,000	1,000 - 8,000	8,000 - 18,000
Flux (Gallon per square foot per day) ***Note**: 1 GFD = 1.66 L/m^2/hr*	50-100 gfd	20-30 gfd	5-8 gfd	8-20 gfd
Backwash	Not Required	10Q	2Q	Membrane Damaged if backwashed
Configuration	Skid Mounted	Skid Mounted	Membrane Tank	Membrane Tank
System Energy	4-6 KWhrs /m^3	0.4 Kwhrs / m^3	0.4 Kwhrs / m^3	0.4 Kwhrs / m^3
TMP (pressure per square meter at gauge)	10-50 PSIG	1-3 PSIG	7-10 PSIG	1-3 PSIG

Freely adapted from Dynatec Systems. (http://dynatecsystems.com/index.asp?PageID=126 (Accessed 19 October 2012)).

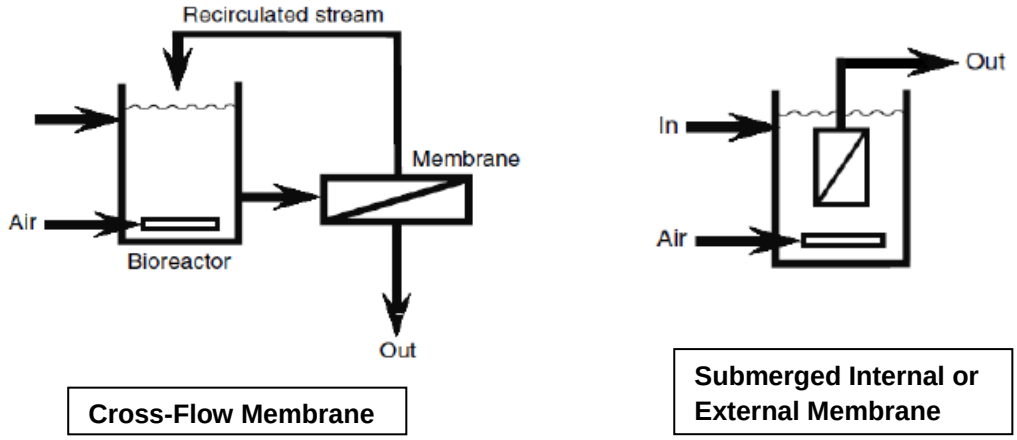
[(a)] Pouliot *et al.,* 2008. (**MF**: Microfiltration **> 0.1µm**; **UF**: Ultrafiltration **1 - 500 nm**; **NF** : Nanofiltration **0.1 - 1 nm**)
[(b)] Polymeric: cellulosic, polysulfone, polyamide; inorganic: ceramic, carbon-supported zirconium oxide, stainless.
(*) For information, **RO**: Reverse Osmosis **<0.1 nm**; TMP between 3.0 and 5.0 **MPa**, use for salt filtration (Pouliot *et al.*, 2008)

2.5.9.2 Treatment process

Mixed liquor suspended solids (MLSS) concentrations of 12 – 15 g/L (4 to 5 times conventional Activated Sludge) are recommended for submerged MBRs (Melin *et al.,* 2006). There two basic configurations for membrane bioreactors (see Figure 8). The submerged membrane bioreactors, inside of the biological tank, and the side-stream membrane bioreactors where the biomass is pumped to the membrane and recirculated back to the biological tank. Side-stream membranes are preferable in industrial wastewater treatment when the conditions such as shock organic loads, high pH or temperature are more variable (Yang *et al.,* 2006).

An external submerged bioreactor is easier to clean avoiding permeation of chemical cleaners protocol requires it. The MLSS is pumped into the external chamber and the retentate is returned to the main bioreactor as with other types of side-stream processes. This configuration helps maintenance cleaning or replacement modules while the bioreactor is still processing (Smith *et al.,* 2012).

Figure 8: Sidestream and submerged membrane bioreactor configurations.



MBR hydraulic retention time (HRT) and solid retention time (SRT) are independent from each other, which allows the process to run faster and at a higher MLSS concentration. This may help to control the sludge yield but certainly increases efficiencies per m^3 of reactor (Brindle and Stephenson, 1996a).

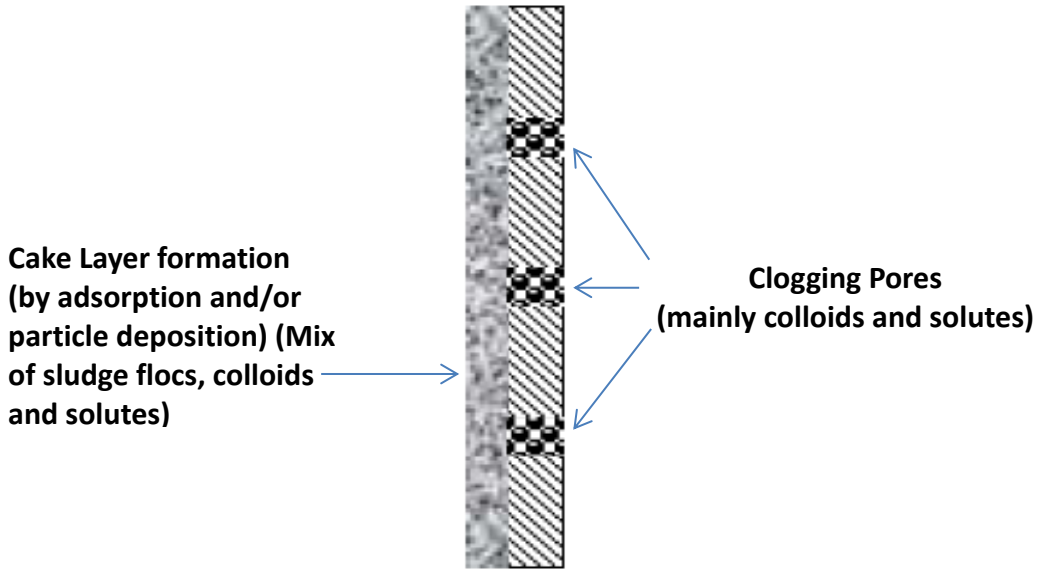
2.5.9.3 The Fouling

Membrane bioreactor technology has been used for a couple of decades, but has not yet become common in the market due to the operational costs caused by power consumption and fouling (Naessens *et al.,* 2012). According to Zhang *et al.*, (2006), fouling is the major issue, thus many studies have been made to better understand mechanisms of fouling and fouling control strategies in AeMBR. The operational process costs are caused by cleaning chemicals and flow reversals which get more frequent with gradual deterioration shortening the membrane lifetime expectancy (Le-Clech *et al.,* 2006). Common strategies put in place to control the fouling depend on the membrane configuration. In external cross-flow configurations, a high velocity cross-flow is maintained to limit foulants building up on the membrane. In submerged configurations, fouling is controlled by sparging, backflushing, and/or membrane relaxation (Smith *et al.,* 2012). Chemical cleaning is made by periodic soaking or flushing with NaOCl, H_2O_2 and HCl, which are the most conventional chemical combinations.

The concentrations can be made depending on membrane characteristics (material, pore size, hydrophobicity or hydrophilicity and roughness), fluid characteristics (feed composition, floc properties and biomass activity) and operational conditions (both biological and hydraulic) (Le-Clech *et al.,* 2006).

The fouling is from the accumulation of organic and inorganic materials reducing membrane permeability which demands either greater pressure to drive the liquid through the membrane or more regular cleaning. Three main types of fouling are recognised affecting the membranes: feed and biomass characteristics, membranes properties and MBR operating conditions (Le-Clech *et al.,* 2006, Judd 2006, 2011). Figure 9 shows how the membrane can be clogged by adsorption of soluble microbial products (SMP) at the surface, pore clogging (material becomes stuck inside pores) and particle deposition (simple accumulation of materials on the walls a cake) (Liao *et al.,* 2006). The membrane channels (inside the membrane) and the aerator ports are also reported to be obstructed (Judd, 2011). The most difficult parameter to control is probably the nature of the biomass matrix because it is quite highly dependent on the influent wastewater which changes all the time and the biomass cannot follow the fast changes. Zhang *et al.* (2006) state that the membrane properties and the hydrodynamic environment experienced by the membrane, are the two most common factors that contribute to fouling and which are the easiest to manage by the operator.

Figure 9: Membrane Fouling



2.5.9.4 Effect of HRT and SRT

Performance and membrane fouling are HRT and SRT dependant. Low HRT reduces the size of the plant and thus its footprint whereas a high SRT improves the treatment achieved, by increasing the biomass. Low temperatures and high SRT creates more soluble microbial products (SMP) and extracellular polymeric substance (EPS) which,increases the fouling rate. Microbial materials includes extracellular carbohydrates, proteins, lipids and nucleic acids (Smith *et al.,* 2012).

Many studies (Bouhabila *et al.,* 2001, Meng *et al.,* 2006a, Itonaga *et al.,* 2004) state that SRT and HRT should be dependent on colloidal fouling (particle deposition and/or pore clogging). Bouhabila *et al.* (2001) tested a membrane (type Zenon HF) with feed made of synthetic dairy wastewater and reported a flux of 12 $L.m^{-2}.h^{-1}$.

The HRT was 3.3h and the SRT varied between 10, 20, 30 days. They recorded that membrane efficiency decline was proportional to the amount of colloids present which was inversely proportional to the SRT.However, Smith *et al.,* (2012) reported on a study from Baek *et al.,* (2010) who observed a decrease in EPS concentrations at higher SRTs, suggesting that increased biomass concentrations reduced adsorption fouling but the excess biomass created more particle problems from cake layers.

2.5.9.5 Membrane performance

The performance of a membrane is dependent on the efficiency to filter the organic matrix (from macromolecules to suspended solids). Darcy's law is defined as the rate of which a fluid flows through a permeable medium, and thus is usually the common starting point for model equations for filtration. The following model relates membrane flux (as setting) to the measured trans-membrane pressure (TMP), using constants for the sludge viscosity, temperature with or without solids dependent parameters (Naessens *et al.,* 2012). The simplest theoretical approach for the permeate flux is the resistance-in-series model (RIS) (Chang *et al.,* 2002) (1) (2)

$$J = \mathrm{TMP}/(\eta.R_t) \qquad (1)$$

$$R_t = R_m+R_c+R_f \qquad (2)$$

J is the permeate flux ($L.m^{-2}.h^{-1}$); η is the dynamic viscosity of the permeate (Pa.s); the trans-membrane pressure (TMP) (Pa); R_t is the total resistance to filtration (m^{-1}); R_m is the intrinsic membrane resistance (m^{-1}); R_c is the (reversible or external) fouling resistance caused by the cake layer deposited over the membrane surface (m^{-1}); and R_f is the (irreversible or internal) fouling resistance produced by adsorption of dissolved matter (pore narrowing) and/or pore blockage within membrane (plugging) (m^{-1}) (Chang *et al.,* 2002, Guglielmi *et al.,* 2007). Therefore the total resistance is the combined effect of the clean membrane resistance R_m and summation of fouling mechanisms reducing filtration rates and increasing the TMP which is proportional to the resistance. Separate models are required for the resistance components usually differentiating the resistance coming from the cake layer and the pore blocking (Naessens *et al.,* 2012). An overview describing some of the different membrane resistance approaches in the literature is summarised in Table 7 from Naessens *et al*., (2012).

Table 7: Overview of the different possible decompositions of the filtration resistance used in literature

Reference	Resistances in series decomposition	Partial resistance models
Broeckmann *et al.*, 2006	$R = R_m + R_c + R_p + R_{irr}$	R_m : Darcy's law R_c : Broeckmann et al. (2006) R_p : Broeckmann et al. (2006) R_{irr} : Wintgens et al. (2003)
Busch *et al.*, 2007a	$R = R_m + R_c + R_p + R_b$	R_m : Darcy's law R_c : Broeckmann et al. (2006) R_p : Broeckmann et al. (2006) R_b : Busch et al. (2007a)
Drews *et al.*, 2009	$R = R_m + R_c + R_{sta} + R_{com} + R_{int}$	R_m : Darcy's law R_c : Chudacek and Fane (1984); Elmaleh and Ghaffor, 1996 R_{sta} ; R_{com} ; R_{int} : Hermia (1982)
Khan *et al.*, 2009	$R = R_m + R_c + R_f$	R_m : Darcy's law (clean water) R_c : Darcy's law (at end of R_f : Darcy's law (after cake layer
Li and Wang, 2006	$R = R_m + R_p + R_{cd} + R_{cs}$	R_m : not mentioned R_p : Bowen et al. (1995) R_{sf} : Li and Wang (2006) R_{sc} : Li and Wang (2006)
Ludwig *et al.*, 2011	$R = R_m + R_c + R_f$	R_m : not mentioned R_c : Ludwig et al. (2011) R_f : Geissler et al. (2005)

(R_m : clean membrane resistance, R_{sta} : standard pore blocking resistance, R_{com} : complete pore blocking resistance, R_{int} : intermediate blocking resistance, R_p : pore blocking resistance, R_{irr} : resistance by irreversible fouling, R_{cd} : dynamic sludge film resistance, R_{sc} : stable sludge cake resistance, R_f : fouling resistance, R_b : biofilm resistance).

2.5.9.6 Membrane costs

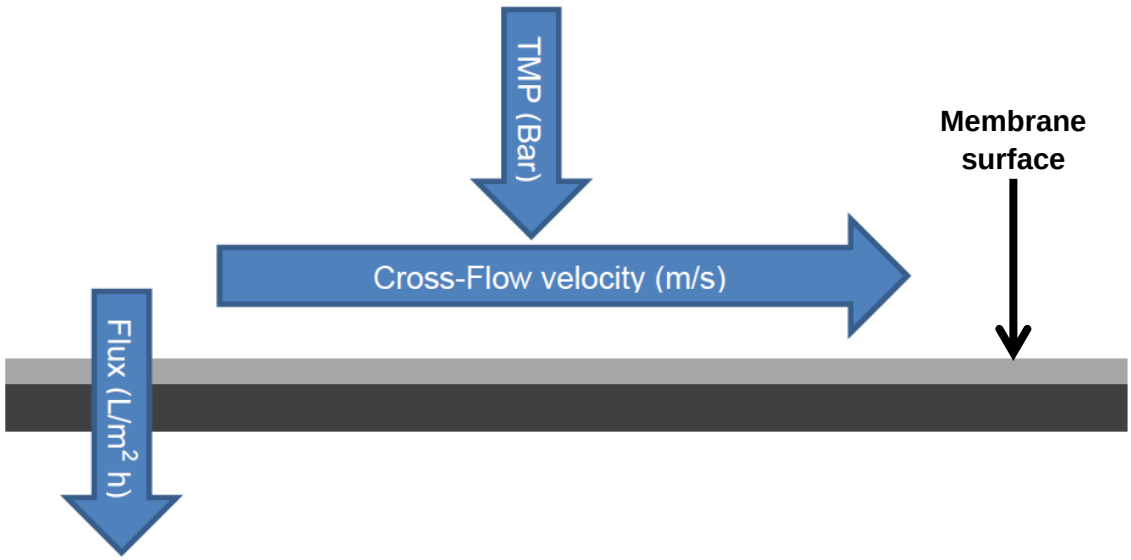
The review of the literature has highlighted the need for wastewater treatment to be more efficient and sustainable in future. Membrane plants are more efficient to fully retain suspended solids as well as low microbial contamination of the effluent (Pinnekamp and Friedrich, 2006, 2009). Their smaller size reduces resource consumed during construction but their energy and chemical use increases the resources used during operation. These additional operation and maintenance (O&M) procedures due to fouling including chemical cleaning also have negative consequences on capital and operational expenditures.

Extra equipment is required, for example, reduction of the pre-screening size (0.5 mm instead 2 mm in conventional fine screen) extra blowers, pumps and periodical membrane replacement (Pinnekamp and Friedrich, 2006, 2009, Le-Clech, 2010).

Attempting to compare published MBR running costs was complicated as the energy consumption may be expressed as volume of treated wastewater (kWh m^{-3}), per membrane area (kWh m^{-2}), per design population equivalent of the plant (kWh/$PE_{equivalent}$), or per pollution removed (kWh/$PE_{removed}$).

Overall MBR are operated with a permeate flux ranging from 10 and 25 L/m^2h, with a TMP of generally less than a half a bar, (Le-Clech, 2010). In relative terms this has been reported to give the energy demand of MBR plants as two to four times higher than that of conventional plants (Novotny, 2010). The higher energy demand of MBR is mainly due to the need for an energy intensive cross-flow (CF) or aeration of the membrane modules to reduce both membrane fouling and maintain satisfactory hydraulic conditions (Figure 10) (Palmovski *et al.,* 2010). The literature reported average values for the specific energy consumption of MBR plants range from 0.64 to 2.3 kWh m^{-3} whereas conventional activated sludge plants (CAS) have an average specific consumption from 0.28 to 0.55 kWh m^{-3} (Novotny, 2010; Brepols, 2011).

Figure 10: Driving forces and parameters adapted from Huyskens (2012)



The extra energy costs are more than 50% of the running costs (Novotny, 2010). As mentioned earlier the pumping for liquid cross-flow and or air scouring for fouling prevention are the costly items.

Chemical and physical cleaning is also required which will differ in MBR plants according to the wastewater treated. For example there are differences as when to start chemical cleaning, the sequence of cleaning steps, cleaning frequencies and chemicals to be used.

Most of these variables are dependent on the nature of the contaminants to be removed, the nature of membranes (hollow fibres, flat membranes, UF, MF), and tolerance to physical back-flushing and high rates pumping (Pinnekamp and Friedrich, 2009, Brepols, 2010, Le-Clech, 2010).

Other costs may be incurred when throughput is reduced during membrane cleanings by reducing the permeate volume, the cost-efficiency of the process decreases. The optimisation of the pore size between the high initial permeability from MF and more easily recoverable fouling in UF is a difficult design decision when selecting MBR application (Le-Clech, 2010)

2.5.9.7 MBR technology for selective results

No literature was found describing the impact of MBR on either nitrogen or P balance, presumably because of the high solubility of these nutrients in final effluents.

As noted MBR wastewater treatment plants are now commonly found in both anaerobic and aerobic configurations.

1 .Anaerobic use of membrane:

This AnMBR configuration has typically been used for high strength wastewater to retain the slow growing methanogens. However, it has also become attractive for low strength (i.e., municipal wastewater) in warm climates since it operates independently in relation to the retention times, enabling the high organic load. (Lin *et al.,* 2009, Le-Clech 2010)

Anaerobic treatment gives two big advantages of an aerobic treatment (Arros-Alileche *et al.,* 2008):

- First advantage: the sludge production is five time less (≤0.07 kg VSS/ kg of chemical oxygen demand) after conversion. Therefore there is less sludge to manage.

- Second advantage: anaerobic digestion converts organic matter to methane and can achieve degradation (conversion of organic matter over 78%) energy yield are 0.2 -0.4 m^3 CH_4 / $kg_{converted\_COD}$ (Demirel *et al.,* 2005)

2. Aerobic use of membrane

This configuration is more common because (Bick *et al.,* 2009; Le-Clech 2010):

- The air scouring reduces the fouling by reducing the probability to attach to the membrane surface during filtration;
- Maintains a high mixing of the suspended solids liquor;
- The possibility of decoupling the HRT and SRT, enables the SRT to be increased and allow slow-growth micro-organisms such as nitrifying bacteria to attain larger populations.
- Addition of chemicals to reduce the nutrients;
- COD removal efficiency is between 95% and 99%.

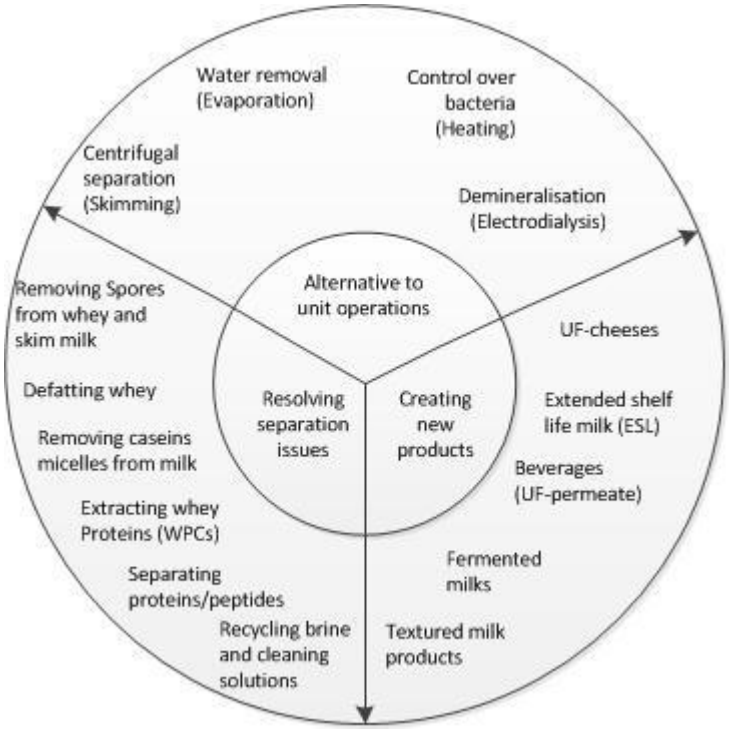
For the purposes of this research, the advantages of the immersed membrane in the aerobic tank were more suitable due to:

- The complex solids nature of the wastewater treated (glucose or milk mixed with settled sewage);
- The process was easier to build and design at laboratory scale;
- Full scale data was available from the Masters thesis preceding this project
- An objective was to investigate nutrients removal (P and N) using milk proteins and calcium.

2.5.9.8 Membrane use in milk industries.

Since the thesis will use the milk to increase the effluent strength, it was also interesting to see how the dairy industries are using the membranes to extract elements from milk in attempts to create new products and markets. The development of membrane science has helped its integration by improving processes in dairy technology.

Figure 11: Membrane applications in dairy industries (Pouliot, 2008)



Dairy processes

Membrane use is summarised in Figure 11 (Pouliot, 2008). Membranes processes are efficient to separate minor but potentially valuable compounds such as bioactive peptides, growth factors and oligosaccharides from milk, whey or fermented dairy-based media.

Summary

As a result of the literature review a submerged membrane reactor was selected for this project for a number of reasons:

- Firstly because the nature of the wastewater was likely to promote poor settling as referred to in section 2.5.5. concerning stronger wastewaters
- Secondly physical models of settling tanks are difficult to build and finally the added milk proteins and calcium complexes are large and separation could be enhanced by membranes.

2.6 Feeding additional carbon to enhance nutrients removal

2.6.1 Introduction

There are many reports from around the world on the impact of weak influent wastewater on the performance of EBPR, these were not encountered when the process was originally developed in the dryer South Africa. (Horan *et al.,* 2009; Ge *et al.,* 2010; Peng & Ge, 2011) All have concluded that EBPR process efficiency is lowered by diluted sewages. Under well controlled pilot plant and laboratory systems it was quickly shown this could be overcome by minimum concentrations of easily metabolisable carbon to phosphorus when EBPR works very well. The PAO microbial community fail to grow when the influent is weak (Cao *et al.*, 2012) because they have to compete for less available VFA against other groups of heterotroph bacteria. Dilute sewage has been shown to lead to a general reduction in microbial diversity and thus weakens the bacterial community (Lie & Welander, 1997; Peng & Ge, 2011).

Many studies have therefore been carried out to compare the effect of different supplementary carbon most has used VFA since these are formed easily in anoxic wastewater. However, despite the number of experiments carried out in the literature, neither VFA or simple carbohydrate additions to full scale plants have been reported probably because it is impractical. In this research, more sustainable and therefore non-VFA carbon sources were compared, one was a mix of carbohydrates mainly glucose and the other, more complex, the milk.

2.6.2 Glucose as external source of carbon:

2.6.2.1 Glucose use solely

Numerous researches have been investigated using glucose and most have found that successful EBPR could not be achieved when using glucose as the sole carbon source.This was due to the dominating growth of non PAO organisms namely GAOs Kargi *et al.* (2005), Wang *et al.* (2010). These bacteria take up glucose more efficiently and more rapidly by being favoured in the way that GAOs assimilate glycogen, poly-P bacteria are then overwhelmed in the anaerobic phase inhibiting orthophosphate release (Mino *et al.,* 1994). Randal *et al.* (1994) however stated that glucose would be detrimental to the EBPR process unless it was first converted into short chain volatile fatty acids (SCVFAs). The PAO's are only able to consume SCVFAs (Lie & Welander, 1997; Hollender *et al.,* 2002 Gebremariam *et al.* 2012).

A detail study on glucose as sole external carbon source was made by Jeon & Park (2000) postulated a theory which would explain some of the contradictions in the research that EBPR was affected by the type of carbon source. Chuang *et al.* (2011) for example contradicted the negative effect of glucose by stating that in their study, the addition of glucose increased the P release during the anaerobic phase and increased the P uptake in the aerobic phase. Jeon and Park (2000) suggested that the release of ortho-P and PHAs production was more related to total TOC than the type of carbon source. They described the possibility of intervention of acid producing organisms (e.g., lactate producing organisms LPOs) when substrate was in excess. These bacteria accumulate glucose as glycogen but then convert it into lactate polymers, which the PAOs are able to convert into PHAs by consuming the poly-P. Thus, the rapid reduction of glucose in the anaerobic conditions did not release orthophosphate because other processes took place first such as fermentation and therefore, reduced the potential amount of PHAs synthesized by the PAOs.

2.6.2.2 Glucose with VFA and non-VFA as external source of carbon

Thus a generally applicable theory was that glucose created instability in the EBPR process because free VFA were not released into solution Randal *et al.* (1997), Akin & Ugurlu (2001). Pre anaerobic fermentation was therefore recommended to promote acidogenesis to overcome this problem (Sudiana *et al*.,1999; Jeon & Park, 2000). Kargi *et al.* (2005) performed laboratory experiments testing the effects of different glucose ratios in combination with organic fatty acid additives such as acetic acid, butyric acid, propionic acid and citric acid on P release and uptake. When glucose as co-substrate did not exceed 50%, their results showed improvement in P removal despite the presence of GAOs. They also found that among the carbohydrates (e.g., fructose, lactose, starch) glucose was actually the most suitable to induce and maintain EBPR.

Acetate is the VFA most commonly used in combination with glucose because propionate is competitively taken up by the GAO strain most commonly found in wastewater treatment plant (Oehmen *et al.,* 2005b,c, 2006a). Hollender *et al.* (2002) compared acetate alone with glucose and acetate combined, and the results showed that the P removal efficiency was best with acetate alone, and the study showed a significant reduction in P release with a worse PHA accumulation when glucose was used as the sole carbon source. Work on mixtures by Gebremariam *et al*. (2012) with glucose and acetate on the other hand brought the best results as a 50:50 mixture than their control reactor fed with acetate only. In contrast, Chuang *et al.* (2010) as noted previously reported worse P release using just acetate rather than glucose.

2.6.2.3 Glucose conclusion.

Using glucose solely or associated with other easily assimilable organic matter (AOM) compounds to enhance EBPR process has brought contradictory conclusions. It is suggested this is as a result of the rapid interconversions of this widely used substrate.. Some of the research, such as Gebremariam *et al.* (2012), artificially adjusted the pH to increase VFA and limit the influence of particulate COD. More widely used has been pre acidification in a side stream, anaerobic reactor to promote VFA production. Thus it was concluded that glucose only accelerates EBPR when it formed VFA or was in combination with acetate, and that acetate on its own is better than glucose (regardless of the ratio) to increase efficiency.

A further conclusion from a critical assessment of the literature was that much of the results and conclusions could be specific to the individual experiments and there was uncertainty concerning general applicability. All of the experiments reported in the literature were carried out at laboratory scale using synthetic sewage usually in sequenced batch reactors. The practicality of using this type of reactor or adding a fine chemical such as glucose or VFA at full scale remains in doubt although acetate has been used to provide an organic source for de-nitrification at drinking water plants. Therefore it was concluded that effluents from food processing would provide a more sustainable source of extra and assimilible carbon. Milk processing offered a good possibility because of its widespread use in northern Europe.

2.6.3 Milk as external source of carbon

2.6.3.1 Introduction

As noted most research has been carried out using VFA, such as acetate (Canizares *et al.,* 1999; Gebremariam, *et al.*, 2012), butyric acid, propionate and formate (Broughton *et al.,* 2008; Ahn *et al.,* 2009) to understand the fundamentals of increasing phosphorous removal. Work has as reviewed in the previous Section also reported on, glucose alone and not reviewed so far in combination with peptone (Canizares *et al.,* 1999). Milk has previously been suggested but for example could increase the level of P content and make the situation worse (Fernandez *et al.,* 2011). Moreover, the literature review has confirmed that dissolved macromolecules cannot directly be assimilated by the PAO (bacteria only capable of taking up small molecular weight organic compounds) unless these molecules are first hydrolysed (Ubukata, 1997; Coulibaly *et al.,* 2003).

Milk was chosen as the supplementary carbon source in this thesis because it was known from site work that it did quickly degrade under both aerobic and anaerobic conditions. Thus the main reasons for researching milk were:

1. During anaerobic fermentation, all the polymers (proteins, polysaccharides and fat) that compose milk will be decomposed by hydrolysis into simple monomers (hydrolases, proteinases, lipases and amylases already present in the milk) (Ubukata, 1997)

2. Fermented dairy wastewater generates a mixture of volatile fatty acids predominantly lactate, acetate, propionate and butyrate (Broughton *et al.,* 2008)

3. Milk contains a large amount of P without being destabilised and this supersaturation makes milk even more interesting. Specific agents for calcium and phosphorus whose mechanisms could be understood offer potential specific ligands for calcium and phosphorus (details in literature review, Section 2.6.3.2).

4. A specific study has been made on several agro-industrial wastewaters as external carbon source and their long-term effect have been assessed by Fernandez *et al.* (2011). They were found that to improve EBPR for a short period of time and most of tested wastewaters were suitable. Wastewaters coming from tomato-processing and milk-bottle industries were suggested for long term dosing based partly on their high ratio COD/P, but little research was completed on the detail mechanisms of action. (Detailed study in the literature Section 2.6.4.2)

5. Another important aspect is the amount of dairy activity in Europe resulting in a large potential availability of dairy process wastewater which could be locally available for EBPR and more cost effective than chemical dosing. Dairy wastewater however, as well as the expected proteins, carbohydrates, fats and organic acids will contain cleaning agents including phosphoric acid (Ahn *et al.,* 2009).

2.6.3.2 Biochemical Characteristics of milk

2.6.3.2.1 Introduction

The white turbid appearance of raw milk is due to the non-settling colloidal suspended particles in a solution called lactoserum. The composition varies throughout the seasons, and depends on the feeding. Total solids in milk have a range of about 11.5% to 18.2% composed as fat (3.0% - 7.0%), protein (3.0% - 5.0%), lactose (4.7% - 5.3%) and minerals (0.75% - 0.80%). The natural pH of milk is in the range of 6.6 – 6.8 (Harper & Marshall, 1984). Amiot et *al.* (2002) gave a summary of the general composition of bovine milk, this is shown in Table 8.

Table 8 : General bovine milk composition (Amiot et al., 2002)

	Maximum Range (%)	Mean Value (%)
Water	85.5 – 89.5	87.5
Lipids	2.4 – 5.5	3.7
Proteins	2.9 – 5.0	3.2
Carbohydrates	3.6 – 5.5	4.6
Minerals	0.7 – 0.9	0.8
Other in traces : Enzymes, vitamins, dissolved gas …		

2.6.3.2.2. Complex relationship of phosphorus and milk

It is possible to exceed the water solubility of phosphorus and calcium in the milk by organic chelates and ligands (See Figure 12 and the balance between the soluble salts shown in Table 11).60% of the total phosphorus in milk is organic phosphate which 50% is bound to the protein caseins The other 50% organic is in solution bound to small molecules such as pentoses, hexoses, glycerol, serine and nucleotides (Gaucheron et al., 1996). Roughly 20% is bounded to the hydroxyls group of amino acids and the remainder is shared between phospholipids and the esters soluble in water. A summary of the inorganics in milk is shown in Table 11 and the organic distribution is shown in Figure 13.

- Fat

The fats in milk are a mixture of triglycerides (98% - 99%) and phospholipids (1% - 2%) which compose typical fat membranes (Grappin & Ribadeau-Dumas, 1989; Harper & Marshall, 1984). The fat is present as large globules (diameter 2 to 12 μm).

- Proteins

Proteins are divided in two major categories caseins and whey:

1) The casein proteins

1.1 Casein characteristics

Raw milk contains four major types of casein: α_S-casein (α_{S1}-casein and α_{S2}-casein), β-casein, κ-casein and γ-casein. They represent 80% of the total protein (Harper & Marshall, 1984). The differentiation is based upon their charge distribution and their sensitivity to precipitate with calcium. Their physicochemical characteristics are presented in Table 9 (Brulé *et al*., 1997). The capacity for the caseins to bind calcium reduces in the following order α_{s2}> α_{s1}> β> κ, which corresponds to their decreasing concentration of phosphoseryl. The phosphate group from phosphoseryl are the main binding sites (situated at the N-terminal region) for the calcium to chelate the phosphate (Brulé *et al*., 1997).

Table 9 : Physicochemical Characteristics of caseins (Brule et al., 1997)

	α_{S1}-casein	α_{S2}-casein	β-casein	κ-casein
Amino acids residuals*	199	207	209	169
Molecular weight	23 600	25 200	24 000	19 000
Phosphoseryl Group*	8-9	10-13	5	1-2
Carbohydrates	-	-	-	+
Calcium sensitivity	++	+++	+	-

* number by molar weight

Phosphopeptides form polar N-terminal regions made from clusters of phosphorylated seryl groups. These phosphoseryl clusters have been hypothesized to be responsible for the interaction between the caseins and calcium phosphate that lead to the formation of casein micelles. The casein phosphopeptides stabilize calcium and phosphate ions through the formation of these complexes. Although the phosphoseryl cluster is pivotal to interaction with calcium and phosphate, other factors are also important. In particular, calcium binding and calcium phosphate stabilization to other casein chains is influenced by peptide net charge, length, and sequence (Cross *et al.,* 2005). Organic phosphates are also covalently bound into the casein peptide chains (α_{s1}-B, α_{s2}-A, β-a^2, κ-B caseins) with 8, 11, 5 and 1 phosphoseryl residues per molecule respectively (Walstra and Jennes, 1984). Caseins and salts in bovine milk are distributed as below in Table 10, they form circular or globular structures or micelles.

1.2 Micelles characteristics

The micelles in milk are round clusters of proteins (α, β, κ caseins) which are able to form larger clusters via calcium phosphate bridges. Micelles are suspended in milk because it is believed that the amphipathic, glycosylated C-terminal end of k-casein protrudes from the micelle surface, form a so-called "hairy layer" that sterically stabilizes the components and thus, prevent them to either collapse or coagulate (Holt *et al.,* 1996). The sizes of the micelles are from 20 to 600 nm with an average of 100 nm (Grappin & Ribadeau-Dumas, 1989). The actual model representation of micelles is that they are made of sub-units including 10 to 100 molecules of casein known as sub-micelles (Figure 12). A calcium-phosphate bridge binds them to each other. The composition of the sub-micelles is different in the core (hydrophobic) where β and α_{s1} caseins are in the majority, compared to the peripheral hydrophilic ones (absorbent) where caseins α_{s1}, α_{s2} and κ are in the majority (Amiot *et al.*, 2002).

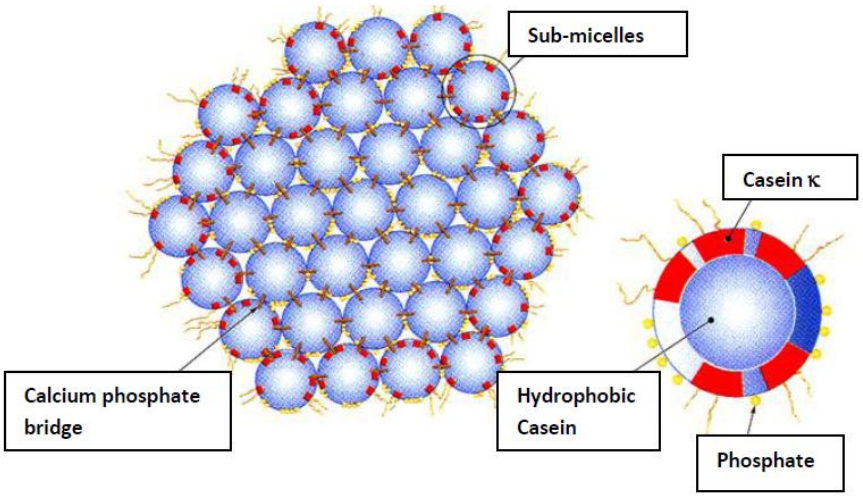
Calcium phosphate is present as nanometer-sized ion clusters, within the caseins but are not covalently bound, but in colloidal associations within micelles and agglomerates (de Kruif, 1999). The micelles are made of 92 % caseins and the remaining 8 % are salts of which the most important are calcium and phosphorus (Amiot *et al.*, 2002). In the casein micelles, the micellar calcium phosphates (MCP) is about 50 % of the inorganic phosphate and plays a major role in maintaining the structure of the micelles which disaggregate when MCP is removed (Walstra & Jenness, 1984).

Table 10 : Distribution of casein and salts in micelle bovine milk (Brule *et al.*, 1997)

Caseins	(g/100g)	Salt constituents	(g/100g)
α_{S1}-casein	33	Calcium	2,9
α_{S2}-casein	11	Magnesium	0,2
β-casein	33	Inorganic phosphate	4,3
κ-casein	11	Citrate	0,5
γ-casein	4		
Total Caseins	92	Total Salt	8

Structures of micelles

Figure 12: Model of casein micelles with sub-units (Amiot *et al.*, 2002)



A) The whey proteins.

Unlike the caseins, the whey proteins are classic globular proteins with a tight tertiary structure, occurring in milk as monomers or oligomers. Two of them are dominant, β-lactoglobulin and α-lactalbumin which represent about 50% and 12% of total whey proteins, respectively. Several other proteins occur in milk: serum albumin, immunoglobulins, lactoferrin, and enzymes such as lactoperoxidase, xanthine oxidase, etc. (Grappin & Ribadeau-Dumas, 1989).

In addition, some non-protein nitrogenous (NPN) substances (ca. 1.5 g/L) are present in milk such as urea, creatinine, ammonia, peptides, nucleotides, vitamins etc. (Ribadeau-Dumas & Grappin, 1989).

3. Carbohydrates

Apart from lactate citrate is the principal sugar (1500 – 2200 mg/L,) 10% is electrostatically linked to the casein micelles (Gaucheron *et al.,* 1996). Citrate has a strong affinity to bind calcium phosphate during their aggregation into ACP (see explanation in nucleation Section 2.6.7.2).

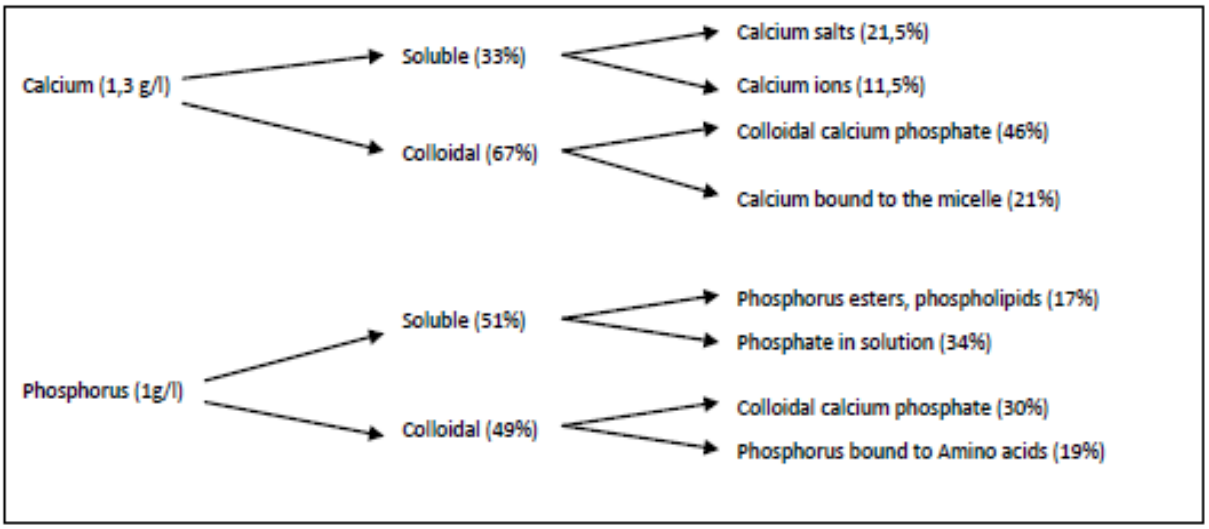
B) The minerals.

The major salts in the mineral component are those of calcium, sodium, potassium and magnesium, which occur in combination with inorganic phosphates (soluble phosphate and micellar calcium phosphates (MCP)), chlorides, nitrates and caseinates (Harper & Marshall, 1984). Table 11 summarises the minerals in bovine milk.

Table 11 : Minerals contents in the bovine milk (Amiot *et al.*, 2002)

Minerals	Content (ppm)	**Minerals**	Content (ppm)
Na	445	**Ca**	1180
Mg	105	**Fe**	0.5
P	896	**Cu**	0.1
Cl	958	**Zn**	3.8
K	1500	**I**	0,28

Figure 13: Distribution of calcium and phosphate in the milk (freely adapted from FAO 1998)



4. Enzymes in milk

Table 12 provides the concentration of some endogenous enzymes in raw milk from cow, goat and human.

Table 12: Endogenous enzymes summary in some raw milk (Park & Haenlein, 2013)

Enzyme concentration and/or activity	Cow	Goat	Human
Acid phosphatase	0.75 U/mL	1.4 U/mL	NA
Alkaline phosphatase	1.8-2.5 U/mL	11-13 mg/L	NA
Lactoperoxidase	30 mg/L 1.5-2.7 U/mL	1.55-4.45 U/mL	1.5 mg/L 0.06-0.97 U/mL
Lipoproteine lipase	0.5-2.0 mg/mL	NA	4-20 mg/mL
Lysozyme	10-35 µg/dL	25 µg/dL	4-40 µg/dL
Plasmin	0.07-0.3 µg/mL	NA	0.07-0.13 µg/mL
Ribonuclease	1000-2000 µg/dL	425 µg/dL	10-20 µg/dL
Sulfhydryloxidase	33 mg/mL	NA	NA
Xanthine oxidoreductase (XOR)	120 µL O_2/h/mL	19-113 µL O_2/h/mL	12 µL O_2/h/mL

Sources: based on data from Chen *et al.,* (2003); Seifu *et al.,* (2005); Fox & Kelly (2006a) and Park & Haenlein (2006).

It was hypothesised by this thesis that the enzymes present, milk would favour the rapid lowering of RedOx potential and help the fermentation:

1. To aid fermentation, therefore as well as being rich in biodegradable proteins, lipids and carbohydrates, milk has endogenous enzymes and thus, inherent capability to to transform these macromolecules into AOM substrates;
2. In the biological RedOx reactions, the transfers of electrons are as important as the transfer of phosphoryl groups. The XOR content concentrated on the milk fat globule membrane (MFGM) exists in two interconvertible forms, the xanthine dehydrogenase (XDH) and the xanthine oxidase (XO), both enzymes reduce molecular oxygen. The XOR monomer enzymes contain Fe_2S_2 RedOx clusters which also include molybdenum and nicotinamide adenine dinucleotide (NAD^+, the coenzyme that XDH reduces into NADH) (Martin *et al.,* 2004 and Park & Haenlein, 2013). The NAD^+ (generally functions in oxidations in catabolic pathways) has a close analog, the nicotinamide adenine dinucleotide phosphate ($NADP^+$) (commonly functions in reductions in anabolic pathways), both are water soluble cofactors that move readily from enzyme to another.

These coenzymes are universal cell electron carriers, (NAD+ and NADP+) using the reversible reduction of the nicotineamide ring. The substrates undergo oxidation (dehydrogenation), giving up two hydrogen atoms. The oxydized nicotinamide of either NAD+ or NADP+ accept a hydride ion and are transformed into the reduced nicotinamide (NADH or NADPH) (Alberts et al., 2008; Tiidus et al., 2012).

Few enzymes can use both, NAD+ or NADP+, most are specific to one or the other. This functional specialisation allows cells to maintain two pools of electron carriers with each pool having its own specific function. The general reactions of these cofactors are

$$AH2 + NAD+ \rightarrow A + NADH + H+$$

$$A + NADPH + H+ \rightarrow AH2 + NADP+$$

A/AH2: are substrates or products (Alberts et al., 2008; Tiidus et al., 2012):

If the ratio [NADPH/NADP] is high [50-100], so that NADPH is in excess, this favours reactions where NADPH acts as hydrogen donor in substrate reduction. This is frequently the case in anaerobic conditions and for the anabolic pathway (unfavourable energetically). In contrast, when the $NADH/NAD^+$ ratio is low (less than 50 making NAD^+ in excess), this favours the co-enzyme working as hydrogen acceptor in substrate oxidation reactions the classic catabolic pathway (energetically favourable reaction).

The half reaction for the reduction potentials are:

a. $NAD^+ + H^+ + 2e^- \rightarrow NADH \quad \Delta\xi° = -0.315\ V \qquad (1)$
b. $NADP^+ + H^+ + 2e^- \rightarrow NADPH \quad \Delta\xi° = -0.320\ V \quad (2)$

$\Delta\xi°$ is the energy difference for the semi-reaction during REDOX (energy of conversion).

Most milk has been heat treated and some enzymes have been identified which survive pasteurisation and ultra-high temperature (UHT) these are the alkaline phosphatase, lipoprotein lipase groups. Plasmins also survive the drying and are still active in powder milk (Park & Haenlein, 2013). Table 13 presents the characterized enzyme regarding their activities and properties.

Table 13: Endogenous heat resistant bovine milk enzymes

Enzyme	Source	Activity	Optimal pH	Properties
Plasmin	Associated with casein micelle	Active on all caseins, particularly on β & α_{s2}	7.5	Contribute to primary proteolysis
Lipoprotein lipase	Associated with casein micelle and in the serum phase	Liberates fatty acids	9.2	Contribute to lipolysis
Alkaline Phosphatase	Associated with phospholipid in MFGM	Active against a wide range of substrates	9.0-10.5	Hydrolyses most bonds phosphate ester and can dephosphorylate caseins

Source: freely adapted from Park and Haenlein (2013)

Summary

The literature review has indicated that 60% of the P in milk is organically bound in colloid particles (micelles and caseins). The literature has also suggested that this P is in the form of non- ionic, non-covalent calcium phosphate bridges holding the structural solids together. It was anticipated therefore that these loose polar associations would be labile during wastewater treatment and contribute to the overall P load, to the detriment of the EBPR process. This was to be an important part of the experimental programme in this research but an important factor was envisaged as being the rate of breakdown of the larger organics into smaller easily assimilible fractions, principally VFA. Previous research on this was reviewed in the next Section.

2.6.4 VFA potential from industrial wastewater

Research was reviewed on the average COD/TOC in domestic wastewaters in Section 2.3. but information is also available on potential VFA production from food processing wastewater for nutrient removal .

2.6.4.1 Organic fraction and the COD for total degradation.

In Section 2.5.8.3 it was explained how PAOs needed scVFA to synthesize PHA using intracellular glycogen by hydrolysing intracellular poly-P with the release of Ortho-P to create energy. This needed to be in anoxic or anaerobic conditions to avoid competition from OHO utilising energetically favourable electron acceptors (O_2, NO_3). In the subsequent aerobic stage, PAOs used the newly produced PHAs to replenish their intracellular glycogen pool whilst accumulating a greater amount of phosphate than released.

Glucose and other sugars are not easily used directly by the PAOs, and it was expected that complex organics in milk would not be used either. A possible scale of rates of hydrolysis was suggested Jonsson el al (2005) to understand the potential to deliver energy when used for BNR..

In Table 14 Jonsson *et al.,* (2005) divided in term biochemical oxygen demand or respiration rate into three to match the IWA ASM model. Fast degradation carbohydrates (e.g., sugars): C-chfd; Medium degradation carbohydrates (e.g., cellulose): C-chmd; Slow degradation carbohydrate: C-chsd, Fat: C-fat and protein: C-protein.

Table 14: COD demand after total degradation.

Formula	Generic name	Total COD / g chfd	COD Demand / 1g C
1 Fast degradable carbohydrates: C-chfd			
$C_6H_{12}O_6$ (Haug, 1993)	Glucose	1g chfd → 1.067g COD	2.62g COD / 1g of C
2 Medium degradable carbohydrate: C-chmd			
$C_6H_{10}O_5$ (Haug, 1993)	Polysaccharides	1g chfd → 1.185g COD	2.67g COD / 1g of C
3 Slow degradable organics: C-chsd			
$C_{46}H_{38}O_{16}$ (Sonesson & Jonsson, 1996)	Lignin	1g chfd → 1.797g COD	2.75g COD / 1g of C
4 Proteins:			
$C_5H_7NO_2$ (Christensen *et al.,* 2003)	Protein	1g protein → 1.42g COD	2.67g COD / 1g of C
5 Fat and oil			
$C_{57}H_{104}O_6$ (Christensen *et al.,* 2003)	Fat/oil	1g fat → 2.90g COD	3.74g COD / 1g of C

Source: Jonsson *et al.,* (2005) – See calculation details in Annex V

Jonsson et al concluded that the more complex the carbon sources the longer will be needed in the anaerobic stage (HRT) to ferment the available carbon to VFA. This is discussed in the next Section.

2.6.4.2 Fermentation time

Success of the EBPR is largely dependent on providing sufficient anaerobic contact time so that the carbon and poly-P metabolisms are able generate sufficient VFAs. However, there is a commonly perceived risk at full-scale, that having too long a anaerobic HRT would lead to VFA depletion and filamentous growth (Tchobanoglous *et al.,* 2003). The fermentation process can be defined as the breakdown of complex substrates into a mixture of simple organic compounds (organic acids, alcohols and other biodegradables substrates) without net oxidation (de Lucas *et al.,* 2007b).

By measuring the fermentation potential (FP) of the carbon source, anaerobic SRT can be more suitably designed and operated with maximum efficiency in order to provide the best yields of easily assimilable biodegradable substrates to the PAO in the anaerobic tank for P release, and in the anoxic tank for NO_x removal. The studies carried by de Lucas *et al.* (2007b) on the potential of agro-food wastewater fermentation by activated sludge from enhanced biological nutrient removal (EBNR) treating domestic wastewater, included several food-process wastewaters. Three among them were applicable to our research, namely, potato (soluble starch), cheese and milk bottle industries wastewaters.

Slaughterhouses and tomato processing industries wastewater were included in the same studies. Most of their experiments were performed in accordance with APHA (1998) phase analysis procedures, using a 0.45 μm pore size filter, to determine the soluble proportion and define the easily assimilable substrate fraction The procedure followed that proposed by the Dutch Foundation of Water Research (STOWA, 1996). The characterisations of these agro-food wastewaters are reported below in Table 15. The biodegradable (organic) COD fractions, SA (soluble fermentable substrates (of particulate substrates after fermentation)) (gCOD m^{-3}) and SF (potential of fermentable products (particulate substrates after fermentation)) (gCOD m^{-3}), were measured by respirometric analysis.

de Lucas et al coined the following equation (8) to compare additional substrates. FP_t is the fraction of soluble fermentation product from an anaerobic time (t) (t(hours) = anaerobic residency time) as a fraction of the total COD of the wastewater. $(S_A)_0$ is the initial concentration of fermentable product (at t=0).

$$FP_t = (S_A)_t - (S_A)_0 / S_{COD} \quad (8)$$

FP_{10} was nominated by de Lucas *et al.* (2007b) on the assumption that after 10 hours of anaerobic stage, most fermentation processes would be finished.

Table 15: Industrial wastewater characterisation during investigation done by de Lucas et al. (2007b)

Parameter		Agro-food industrial wastewater			
Symbol	Unit	CI	MBI	PP	BSP
S_F	gCOD m^{-3}	208	207	183	165
S_A	gCOD m^{-3}	58	29	33	48
S_A/ S_F	%	28	14	18	29
Maximum Fermentation rate	(gCOD $gCOD^{-1}d^{-1}$)	1.5	0.6	3.4	2.1
S_{BCOD}	gCOD m^{-3}	266	236	216	213
S_I	gCOD m^{-3}	85	68	57	86
S_{COD}	gCOD m^{-3}	351	304	273	299

Table 15 was freely adapted - CI: Cheese Industries; MBI: Milk Bottling Industries; PP: Potato Processing; BSP: Beet Sugar Processing – SF: potential concentration of soluble fermentation products; SA: concentration of soluble fermentable substrates formed; SBCOD: soluble biodegradable organic COD concentration (SA+SF); SI: concentration of inert substrates; SCOD: soluble COD concentration

Results from the studies were:

- Cheese and milk bottling industries have the highest potential biodegradable substrates (SF). The maximum percentage not fermented ranged from 71% to 86 % from the MIB and potato industries which were the least efficient to release SA.
- All SF was never entirely transformed into SA during the anaerobic stage. de Lucas *et al.* (2007b) explained that this was due to a lack of suitable enzymes capable of reducing some of the complex structure of substrates.
- BSP and CI have both conversion ratios close to 30% whereas MI and PP are close to 15% despite the fact that BSP and PP (starch) are mostly carbohydrates which were expected to release VFA easier than cheese. Research made by Emrah and Demirer (2010) provided a suggestion as to why this might be in that the higher VFA caused media acidification noting that at neutral pH and constant temperature, cheese was competitive with beet, and milk was comparable to potato;
- The lowest value of maximum fermentation rate was measured for milk then cheese, indicating that the release is slower because the wastewaters with more fat and proteins are more difficult to ferment (the highest value was for the potato wastewater);

- Rate of substrate accumulation (Ra) was higher for the wastewater with greater carbohydrates content that eventually hydrolysed faster into glucose. Under anaerobic conditions, the glycogen accumulation requires less energy from microorganisms than uptake of substrates such as for example PHB from acetate. Dircks *et al.* (2001) provided experimental data to support this observation showing that glycogen from glucose was accumulated 46% faster than the PHB from acetate due to the energy efficiency for storage.

The above review and information and Table 15 using of milk bottling effluents (MBI) should be repeatable if milk is to be used as the supplement. Therefore because of the slower hydrolysis of the fats (Table 14), extra anaerobic time might be necessary to benefit EBPR. There are a number of conclusions that can be made from the review to indicate what would happen when milk is used as a supplementary carbon (de Lucas *et al.* (2005b, 2007b)

- Fresh wastewaters were used for fermentation and each time a new domestic MLSS inoculation. Therefore the microbial ecology (e.g., adapted OHOs) was not acclimatised to the source of COD provided, and thus the PAOs had to use the available hydrolysed VFAs (thus the GAOs could be important within the new sludge used).

  This could have produced biased data for the substrates uptake and for the phosphorus release from the unacclimatised PAOs.

  In this research study, a continuous flow system will be used, keeping the sludge in the anaerobic tank for a long sludge age. Therefore, enzyme and adapted microorganisms will be produced to suit the conditions.
- The highest SF potential was for the milk bottling and cheese industries and in the de Lucas *et al.* (2007b) study, milk fermentation continued for 30 hours, suggesting that milk could provide a slow continuous supply of VFA and reduce the risk of substrates depletion.

A study made by Fernandez *et al.* (2011) agreed that the BNR enhancement using industrial processing food wastewaters was a beneficial economic and environmental option. In their study (using similar food process industries wastewater and domestic wastewater) they extended the work by de Lucas *et al.* (2005b, 2007b) research. They analysed their data in a different way, measuring the phosphorus release instead of respiration rate from food processing wastewater. The rate constant of phosphorus release was measured as (h^{-1}) (k_p) and as a dimensionless ratio of the mg of phosphorus-released per COD stored (Y_{PO4}).

These were compared to P release using only acetate k_p. The parameters have been reported in Table 16.

Table 16: Parameters values for agro-food and reference batch tests (Fernandez *et al.*, 2011)

Wastewater	Phosphorus release					COD uptake	
	$Y_{PO4\ initial}$ (t = 0h)	$Y_{PO4\ final}$ (t = 15h)	β	P_{max} (mg PL^{-1})	k_p (Lg^{-1} $CODh^{-1}$)	COD_{max} (mg COD L^{-1})	K_{COD} (Lg^{-1} $CODh^{-1}$)
Acetate	0.63	0.34	0.41	47.2 ± 2.0	4.2 ± 0.7	141.6 ± 2.6	2.6 ± 0.2
Domestic	0.36	0.33	0.25	24.0 ± 0.6	2.3 ± 0.2	67.0 ± 1.7	2.4 ± 0.2
PP	0.31	0.26	0.19	35.3 ± 2.1	1.5 ± .01	137.2 ± 10.3	1.3 ± 0.2
BSP	0.25	0.15	0.17	18.4 ± 1.1	1.9 ± 0.2	125.0 ± 7.1	1.1 ± 0.2
MBI	0.62	0.23	0.32	12.8 ± 0.2	3.8 ± 0.3	58.2 ± 4.2	1.3 ± 0.3
CI	0.45	0.12	0.30	14.1 ± 0.1	3.8 ± 0.2	119.7 ± 14.7	1.0 ± 0.2

- PP: potato processing; BSP: beet-sugar processing; MBI: milk bottling processing; CI: Cheese industries
- (mean value ± standard deviation); $Y_{PO4:}$ polyphosphate ratio released per g COD stored; β: mean PO_4 released to COD stored over test period; COD_{max}: maximum potential COD uptake; k_{COD}: rate constant for COD uptake.

Fernandez et al came to the following conclusions

- Most agro-food wastewater provided higher rates of P release than using only domestic wastewater;
- Highest *k*p were from milk bottling (MBI) and cheese industries (CI), suggesting that their substrate characteristics after hydrolysis were similar to acetate or VFA acetate was provided at 100%, as a control also used by Lucas et al. 2005b, 2007b);
- The Y_{PO4} followed the same pattern as the *k*p or P release.
- Low Y_{PO4} values were interpreted as meaning accumulating storage pools of poly-P and therefore in the long-term this could reduce EBPR. Ranked by decreasing Y_{PO4} ; were cheese, potato processing and lastly sugar beet wastewaters

An additional experiment for two months was carried out with a restricted analytical programme using 1. domestic wastewater only (DW), 2. domestic wastewater plus cheese industry wastewater (DCI) and 3. domestic wastewater plus milk bottling industry wastewater (DMBI):

- The Y_{PO4}: sequence was DMBI > DW > DCI; it was observed that in the short term EBPR was enhanced for DMBI and DCI, but over extended time and acclimatization the differences in P and COD were better with MBI and DW compared with DCI suggesting that the long term trials would be useful;
- There was an increase in PAO population with MBI wastewater addition (a 37% increase of the VSS) compared with CI wastewater (19% increase of the VSS). This was in accordance with de Lucas et al. (2005b) whose research also indicated wastewaters with a high sugar content had a lower maximum specific growth rate compared to more complex wastewater.

To summarise therefore previous work on additions of milk have shown that the most of the complex organics would be converted to easily assimilible substrate suitable for the PAO. Most of the evidence suggested that milk was a better overall nutrient since it provided other nutrients not available in simpler substrates such as glucose, or wastewaters from beet processing. It was also concluded from the review however that because of the fat content hydrolysis was slower than for other food wastewaters.

2.8 Literature review summary

The awareness of water scarcity is pushing the national and international organisations to protect water bodies and drinking water resources with tougher regulations for a better management and retention of quality.

One of the possibilities is water reuse which allows a faster water cycle and helps to reduce further pollution in waterways. However, perception and quality are issues for the general population aware of the risks of failure of the water treatment plant could be for human health.

Nutrients in wastewater, particularly P, has been shown to be a major impediment to water recycling, thus the EU has introduced measures through the WFD 2003 to restrict the amount of P release. Most wastewater treatment used enhanced biological phosphorus removal (EBPR) in activated sludge since it is commonly recognised to be economic, efficient and sustainable. The luxury P uptake by the polyphosphate-accumulating organisms (PAOs) in the system is possible through different redox phases, usually anaerobic followed by anoxic and then aerobic conditions. Recycling sludge loops and optimal volatile fatty acids within the mixed liquor suspended solids (MLSS) are needed to maintain process efficiency. The disadvantage of the EBPR is mainly due to the competition from other microorganisms for VFA substrates which the PAO metabolic pathway requires. A common competitor is the glycogen-accumulating organism (GAO) which do not participate in P removal. Other upsets or filamentous organisms have also been shown to contribute to the EBPR instability. These two problems in particular have been found to be made worse by dilute sewages common in Europe. A large amount of treated wastewater comes from cities with combined wastewater characteristics (grey and black waters) that was different if storm water was absent or diverted from the wastewater treatment works.

EBPR efficiency has been shown to be closely related to the food to microorganisms (F/M) ratio. Some of the most successful food supplements have been shown to be the VFA such as acetate (HAc) and propionate (HPRr) where they promote P mobility in the anaerobic stage. These have been investigated widely at laboratory scale because these fatty acids are the most commonly found naturally in wastewater treatment plants. However at full scale using predominantly VFA as a performance enhancer would increase the running costs considerably especially to treat at large works where EBPR is common. Therefore, new strategies to promote the development of PAOs but also reduce the competitive GAO growth at lower cost than adding VFA are needed. The literature review has revealed controversies concerning the benefits of complex supplements of AOM to promote BPR.

Glucose has been most commonly considered as an alternative to VFA by several studies because it is cheaper and also easier to be absorbed by microbial populations. Few studies have considered using milk as supplement complex carbon source to enhance EBPR.

The review has noted that milk has a high concentration of calcium and phosphorus, they are found in caseins, between micelles and as free ions without being destabilised despite being at supersaturation concentrations. This complex relationship between the principal minerals makes the milk an interesting carbon source to be considered as a supplementary food. It is likely to be more sustainable than acetate or glucose to improve the EBPR since it is usually locally produced and often wasted. These complexes could promote further amorphous calcium phosphate (ACP) formation from extra organic-inorganic P and Ca ligands. The new larger molecules binding to soluble P may then be more suitable for membrane filtration by increasing the particle size.

Membrane bioreactors (MBR) are used to improve the system reliability furthermore compare to the traditional way of simple gravity liquid/solids separation. It also reduces the WWTW footprint, the smallest particles sizes are kept in the sludge to leave only the soluble elements in the liquid. The concentration of the MLSS can be increased thanks to the total control of the sludge inventory and influence over yield. The main drawback is the fouling, made worse if along with the biological P uptake, chemical additions such as iron salts and alum are used to ensure standards are always met.

Sludge management is very expensive since the waste treatment for metal recovery and recycling is not easy. Using more sustainable lime (calcium compounds) is problematic because of the increase in pH, reducing the EBPR performance.

Calcium can be used in other form such as calcium chloride as it does not cause a damaging rise in pH affecting biomass activity. Moreover species of calcium phosphates (aqueous or solid species) are created within the MLSS could be another advantage with the possibilities of promoting the nucleation of P compound in the sludge and assisting the solids liquid separation process).This has not been previously investigated. EBPR with Ca could improve the waste management sustainability by recycling directly the P sludge for agriculture. The phosphorus bound to calcium is similar to natural phosphate. In this way eutrophication would be reduced further as it is not necessary to process further mineral phosphorus extraction for fertilisers.

In summary there are problems of consistency from EBPR because of weak sewage. This can be overcome by additions of other wastewaters and milk has been tried but rejected because of the additional burden of P from the milk. In this research we suggested this disadvantage might be counteracted by promoting the calcium phosphate complexes in the milk to act as nucleation sites to generate solid P which could be separated by the MBR process.

3.0 Aim and Objectives

As mentioned in the literature research conducted by Fernandez *et al.* (2011) the wastewater from milk industries could be used to provide AOM and P complexing organics in domestic wastewater for long term dosing. The variation of domestic wastewater in COD availability can be an important influence but also because of yearly seasons. Thus an academic assessment of EBPR using milk as external carbon source in domestic wastewaters and how EBPR works as an application for industrial wastewater will be investigated. The usage of calcium chloride ($CaCl_2$) as coagulant has also been reported in the literature, and will be included in the results as an hypothesis to assist and improve for P sequestration during the EBPR process.

3.1 Aim

This proposal is to establish opportunities to reduce chemical use (i.e., supply costs, storage costs, waste disposal cost) for the removal of P whilst complying with the EUWFD and the quality standards which set 95% consent P < 1mg/L transformed into UK Environment Agency (E.A.) requirements for all watersheds.

The academic aim of this thesis was to research bovine milk as AOM for promoting calcium phosphate precipitation by provoking bonds between hypothesised milk chelating agents and soluble P in the wastewaster, using the basic biological phosphorus removal process stream as a model. From an applied research point of view, the EBPR process is now widely used for the treatment of domestic wastewater which has typical P concentrations between 4 and 12 mg PO_4--P/L (Tchobanoglous *et al.*, 2003). Research on the adoption of EBPR for the treatment of industrial and agricultural wastewaters is not common as they can reach high-strength wastewaters and are rich in phosphorus (higher than 100 mg PO_4--P/L (Broughton *et al.,* 2008).

3.2 Scope

Milk wastewater has a strong COD content but is also rich in phosphorus. The high volumes of effluent discharge from the W.W.T.P. would require a lot of chemicals (i.g., iron and aluminium salts) to help the biological phosphorus removal (BPR), and thus have adverse effects on both the life expectancy or damage to the MBR as well as creating more and less easily recycled sludge.

The aim of this research was to improve the sustainability of soluble P removal in the wastewater. The study starts from the hypothesis that some ligands for P both organic and inorganic are present in bovine milk in particular and the ability of these mechanisms to work in wastewater treatment will be assessed empirically, based on the removal of soluble P.

This research has not included identification or speciation of the PAO's by a biometric assessment and the importance of microbial species on P capture nor has it analysed the molecular nature of phosphorus in wastewater (particle size distribution and the chemical characteristics).

3.3 Objectives

1. Review of information about existing biological and chemical precipitation processes for phosphorus (P) removal;
2. Link the soluble P removal with wastewater characteristics that will include preliminary and specific analysis to compare them with earlier work referred in scientific literature;
3. Perform laboratory scale bioreactor experiments to investigate and assess simultaneously the combination of biological, physical and chemical P removal capacity using bovine milk and $CaCl_2$:augmentation.
    a) Compare the effect of extra carbon as milk with traditional sugar sources on P sequestration within the sludge and biomass
    b) Evaluate the total soluble P removal after each different stage of the treatment process and the potential benefits from supplementary $CaCl_2$ dosing.
    c) Find the ideal HRT and SRT for pre-fermentation aimed at synthesis of the maximum RbCOD from the carbon source for the EBPR.
    d) Design the best compatible sequences of phases defined by redox, denitrification and pH to be implemented to improve EBPR performance.

4. Assessment of Kubota® submerged flat sheet membrane bioreactor for enhanced P removal. There was little in the refereed journals concerning any benefits to P removal but there was also little in the other network sources; e.g. EA, DEFRA, BBSRC and the Trade Associations; Information has largely remained with the manufacturers.

3.4 Linking the knowledge gap to the objectives

The preceding Literature review has highlighted a number of poorly researched areas which could improve wastewater treatment for nutrient removal. These have led to the thesis objectives:

1. **Review of information concerning combinations of biological and chemical processes for phosphorus removal. Investigate the use of low doses of calcium chloride;**

There is a wealth of research into phosphorus removal using coagulants; but few investigated calcium as coagulant for P at neutral pH. Most reported work used calcium at high pH to generate a P concentrate for recovery. Food processing wastewater will often contain greater Ca than domestic wastes and their addition to increase the TOC content may have this additional benefit of forming calcium phosphate complexes. $CaCl_2$ for P sequestration within the sludge and for biomass P uptake potential was investigated:

The literature, reported most commonly on coagulation with Aluminium Sulfate (Alum), Polyaluminium Chloride (PAC/PACl), Polyaluminium Sulfate, Ferric Chloride, Ferric Sulfate and polymers. Calcium would be a more environmentally and epidemiologically appropriate chemical.

Hypothesis 1:
A calcium based chemical adjunct will not need any pH adjustment to release [Ca^{2+}], and will not modify the biological activity of the MLSS.

2. Assessment of Kubota® submerged flat sheet membrane bioreactor for enhanced P removal. Little information has been found in either the refereed journals or other networking sources.

Membranes are not often use in domestic wastewater treatment plant due to their operation and maintenances costs. Therefore the coagulation/flocculation followed by settling is the preferred less expensive option. For industrial effluents on the other hand, where the literature review has suggested settlement problems, membranes to remove particulates from wastewater, has been shown to be efficient. However, membranes are prone to clogging with a periodic need for cleaning related to the wastewater treated for example dairy waste. The regular cleaning of membranes would be expected to reduce their life expectancy.

The frequency of cleaning and the resilience of the membrane to the cleaning agents, which can be corrosive, was investigated. It was hypothesised that cleaning might be faster since the literature suggested the bonds between organic particulates and calcium are easier to remove than those formed with iron or aluminium.

3. Link the soluble P removal with wastewater characteristics, routine analysis was used to compare results with earlier work referred to in the literature;

It was concluded that the local WWTW settled sewage would provide all the natural trace nutrients in the organic matter (NOM), and also some complex colloids and dissolved material that a synthetic sewage could not. The Loughborough sewerage system is mainly combined and occasional weak strength was anticipated from rain. This would be compared with previous work that suggests this causes problems for EHB processes.. The University is also an important contributor to the WWTW (33%) and the strength is also seasonally affected by term times. The literature confirmed the local sewage is similar to the European domestic average.

The analysis on the sewage collected included the TOC, pH, turbidity, suspended solids, and nutrients as Total P and Total N concentration. Nutrient analysis was used to track the soluble P and Nitrate through the process. Changes in MLSS and VSS were measured in the experimental plant.

Hypothesis 2

Supplementing real sewage with other stronger effluents was preferential to synthetic additional carbon for enhanced P removal.

4. Laboratory scale bioreactor experiments were carried out to investigate and measure the effect of two different extra carbon source additions.

The literature confirmed that calcium and phosphate are in excess in milk and that they are relatively stable with temperature and pH.

The components in milk might create conjugates that would not be easily biodegraded. Thus, it was intended to investigate whether these calcium-phosphorus affinity properties persisted in WWT to enhance P removal.

There was little literature found which used well controlled laboratory scale continuous flow activated sludge and none with membranes. It was concluded that this approach was easier to build and operate and less confusing for test results than a larger pilot-plant or batch reactor. The fermentation process for the milk degradation was expected to be rapid and integrated in the process without special provision. The starting point of the design was the University of Cape-Town process which then evolved in terms of sequences as the results were generated.

Hypothesis 3

There are chelating agents for soluble phosphorus in bovine milk that could be identified for later isolation and possible future use in the wastewater treatment.

Hypothesis 4

Insoluble calcium phosphate complexes will be created as the calcium ion [Ca^{2+}] is expected to be a strong chelator for soluble P without being detrimental for PAO activity. Milk is predicted to be an ideal nutrient that has the ability to promote the growth of phosphorus accumulating organisms (PAOs, DPAOs) in the mixed culture environment of activated sludge. Therefore biological activity will be improved by the TOC increase without creating more difficulty to achieve the necessary final effluent quality.

Hypothesis 5

It is possible that a process using membrane separation would overcome the usual bulking problems with milk effluents and be applicable at full scale plant for EBPR.

4.0 Laboratory-scale rigs

4.1 Introduction

The first year of experiments were used to develop and design the laboratory scale bioreactors. It was important to be able to have well controlled experiments and characterised materials. Full-scale wastewater treatment plants experience upsets. An upset is an unexpected deviation of one or more parameters in the inlet stream that leads to a partial or complete process failure. The event could be a transient deterioration in general performance treatment that is either temporary or sustained, depending on the type of influent disturbance. These episodes often generate dramatic increase in water contamination levels, which consequently do not comply with regulations. Examples: deterioration as a consequence of unforeseen discharges of chemicals or increase of dissolved organic carbons because of the weather. In order to reduce side down effects, the laboratory reactors were made as large as possible whilst allowing for an individual to control the entire process.

4.2 Choice of wastewater

This research intended to mimic as possible the real conditions, i.e., this included range of nutrients, microorganisms and miscellaneous things usually found in real wastewater. Therefore, as a first decision, synthetic media was not considered. The main advantage of having synthetic sewage is to ease the reproducibility of the data because it brings very stable operating conditions. The literature review has established that there has been little work using real domestic sewage, and using controlled laboratory scale reactors also leads to arguable findings when applied to full scale plants for EBPR improvement (Lopez-Vazquez *et al., 2009*).

To reduce unpredictability due to the daily changing wastewater conditions, the collection was made once every week, and the settled sewage kept in the cold room at 4°C on the assumption that the influent characteristics would remain stable during 7 days of experiments before the next batch.

During tests the total P concentration was then artificially increased in the influent tank by manual additions to the concentration desired. However, the total nitrogen concentration was not increased other than as a consequence of the extra milk additions.

A thorough mixing via two stirrers will keep the particles in suspension as it was when the sludge entered the WWTP system for treatment. This way, the sludge was kept aerated before the influent entered the laboratory rig, and avoided a pre-anaerobic stage. Table 17 shows the characteristics range of the collected settled sewage during the treatment period.

Table 17: Local wastewater sample collected characteristics

Settled Sewage (mg/l)	Range	Settled Sewage (mg/l)	Range
Suspended Solids	170 - 230	**Tot Nitrogen (as N)**	35 - 50
Dissolved	400 - 650	**N-NH_4**	25 - 30
BOD_5	150 - 200	**Phosphorus (as P)**	6 - 12
COD	400 - 650		
TOC	130 - 200		

The inoculum biomass for the MLSS was also collected directly from the aerated tank from the local wastewater treatment works. Every time a new phase of experiments was set-up, then fresh MLSS was collected unless stated otherwise.

4.3 Extra carbon food

A synthetic sewage recipe was originally developed by William & Taylor (1968) and later adapted by Phanapavudihikul (1978). The synthetic sewage water was used as a reference to respect the carbohydrate ratio identified by their authors. The mix would then be used for the control rig glucose to increase the sewage strength with a competitive range of materials to compare with the milk complexity. Table 18 presents the elements that compose the recipe of the synthetic sewage.

Table 18: The recipe of the synthetic sewage

Constituents	Concentration (mg/l)
Dextrin	150
Ammonium chloride	130
Yeast extract	120
Glucose	100
Soluble starch	100
Sodium carbonate	150
Detergent (commercial)	10
Sodium *di*-hydrogen orthophosphate	100
Potassium sulphate	8.3

Modified from the recipe developed by Marquet et al (1999)

From the recipe, soluble starch and dextrin will be used and added to glucose as they are:

- All the components are from the same group of low-molecular weight carbohydrates (starch is a long chain of glucose and dextrin is the hydrolysis product of starch);
- They were selected to be easy to dissolve in water so they can be distributed by peristaltic pump;
- They were also easy to use as batch test additions for short term experiments as described earlier (See Section 2.7.2).

The yeast extract is a complex carbon source and therefore was not considered to be used. Other products were not added as it was considered these nutrients would be in a suitable concentration in the settled sewage collected.

The possibility of testing real milk waste from a local milk producer to take into account seasonal variations was investigated but, it would have been quite changeable and introduced an extra variable into otherwise controllable feed. Thus, the choice was also based on the ease of obtaining a common material, and so therefore long-life semi-skimmed milk buy in local supermarket was used for the experiments. The semi-skimmed milk included appropriate fat content avoiding the potential effects from clogging the membrane with diluted filler fat milk or difficulties for the filtration of samples collected.

The differences between the lines were in the feed enrichment but in order to keep the same strength in the anaerobic zone, the COD was fixed as compared with the milk strength. The glucose mix concentration was made comparable with the milk as follows:

1.0% milk concentration: equivalent to 0.4 g/L glucose – 0.1 g/L dextrin – 0.1 g/L soluble starch (diluted in 300ml tap water).

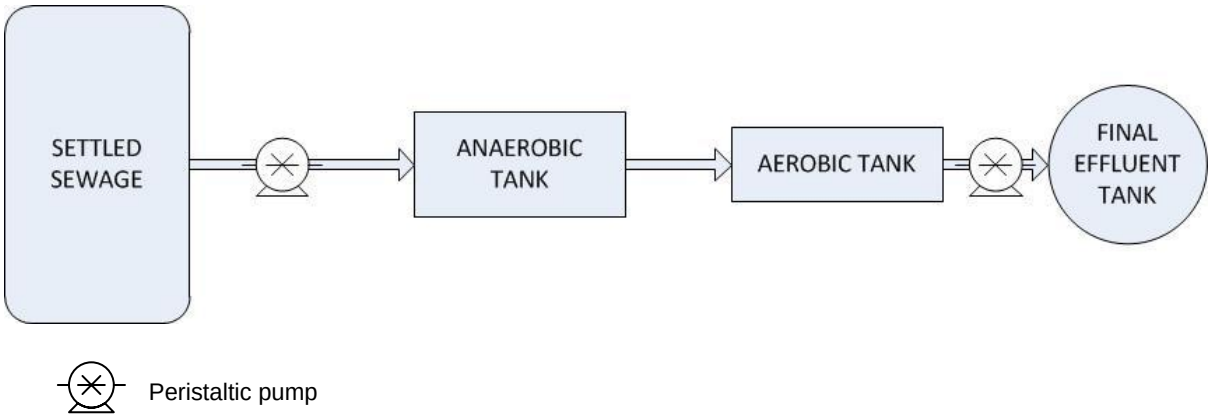
2.5% milk concentration equivalent to 1.0 g/L glucose – 0.2 g/L dextrin – 0.2g/L soluble starch (diluted in 300ml tap water).

4.4 Laboratory scale continuous flow system

A separated series of batch reactor experiments were carried out to rapidly assess calcium complexes for their potential to remove soluble P in wastewater. These batch test trials were inconclusive, and a simple continuous plug flow system was used for all the subsequent reported experiments.

A continuous gravitational flow speed was regulated by a series of peristaltic pumps to control the laboratory scale continuous flow system based on the University of Cape Town process model (UCT). Several different configurations of design (HRT, pumps speed, physical structures…) were tested. The first simulation tests used the laboratory rig configuration, represented in Figure 14.

Figure 14: Initial continuous flow laboratory rig



The laboratory continuous flow system was designed, made and assembled in the Civil and Building Engineering Water Laboratory workshop at Loughborough University. It was decided to work at ambient temperature in an unheated pilot laboratory, and not try to maintain a constant temperature for microorganisms because the aim was also to observe how the performance was affected by warm or cold conditions. The temperature inside the laboratory did not fall below 4^{o}C during the experiments.

Every time a new Phase of experiment was started, the complete system was emptied and cleaned from the previous tests to provide an identical initial starting condition. Several days (usually 7), were needed to stabilise and acclimate the microorganisms to the extra-carbon feed and the $CaCl_2$, after this period of acclimatization, data collection began.

A second and more effective laboratory rig was an upgrade version of the first (Figure 14 above). Continuous minor improvements and adjustments were brought in during experiments as the analysis of the results of each stage process become known.

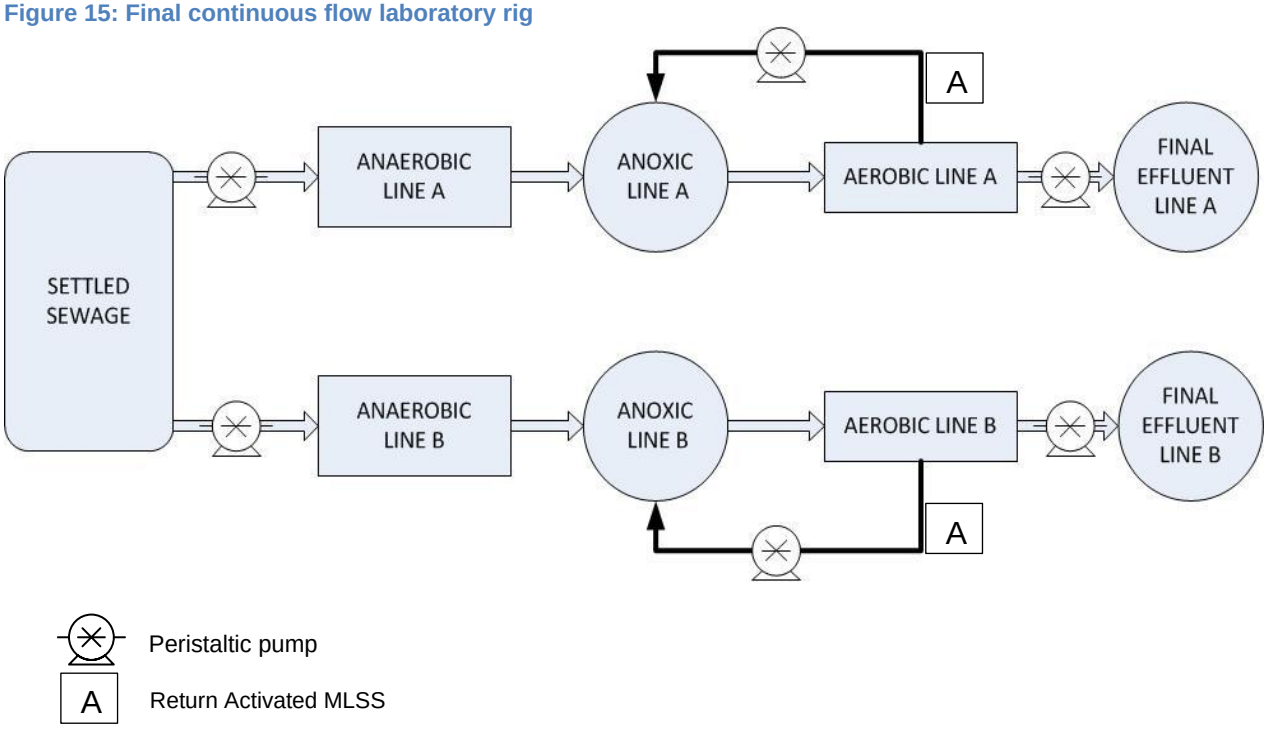
4.4.1 Laboratory continuous system flow charts.

The laboratory bioreactor is designed for the current standard wastewater process for biological P removal (anaerobic-anoxic-aerobic sequence) which has proved to be the most efficient over a long period (Kuba *et al.,* 1997; Zhang *et al.,* 1988). Considering this postulate, our laboratory system was designed as two lines working in parallel in completely identical conditions. This way, it was easy to observe any change in results after a specific test was brought to one line, and compared performances to the unchanged other line.

4.4.1.1 General system flow chart.

Flow chart in Figure 15 describes the circulation process of the 2 lines in parallel operated during the last part of research.

Figure 15: Final continuous flow laboratory rig



The design and the flow remained unchanged as (Figure 15) until the end of the research. The HRTs for each zone and especially anoxic and aerobic tanks were changed to control the NO_3 concentration as nitrate is known to be competitive and detrimental for EBPR performance (Kuba *et al.,* 1994), and thus the nitrate level was monitored to be linked to any negative effect on performance..

The other modifications included:

- Feed concentration;

- Chemical injection zone;

- Chemical concentration.

Unless stated otherwise, usually line B was mostly used as the control line and line A was used as the line testing the modification.

4.4.1.2 Operational description flow chart

Figure 16 describes the operational sequence of a single line. The HRT could be modulated easily either by the pump speed or by increasing (reducing) the capacity within the tank. The adaptability of the laboratory based plant was greater than with a larger pilot or full-scale plant. New modifications and configurations could be instantly done, and microorganisms rapidly adapted, usually over the week-end.

Figure 16: Schematic laboratory rig view

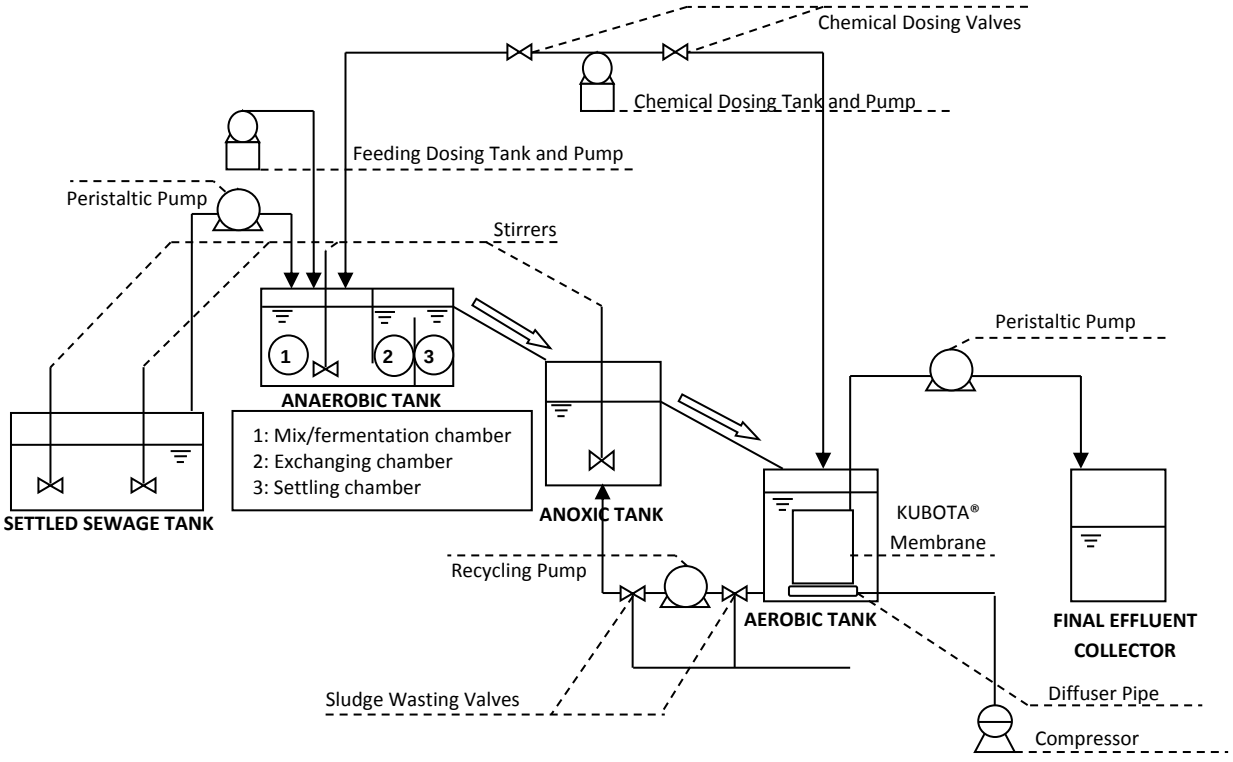


Figure 17 and Figure 18 are the pictures of the laboratory rig used for the analysis during the research.

Figure 17: Laboratory system – From anaerobic tank to final effluent collection.

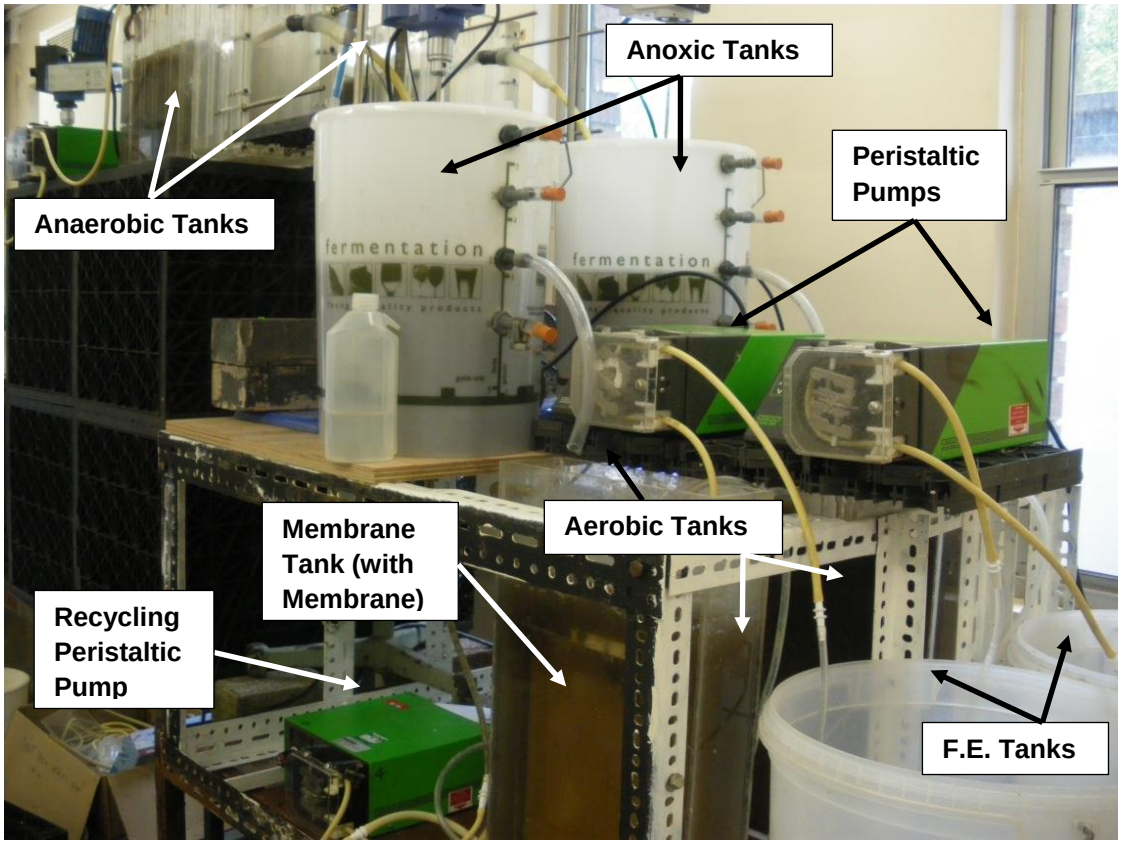
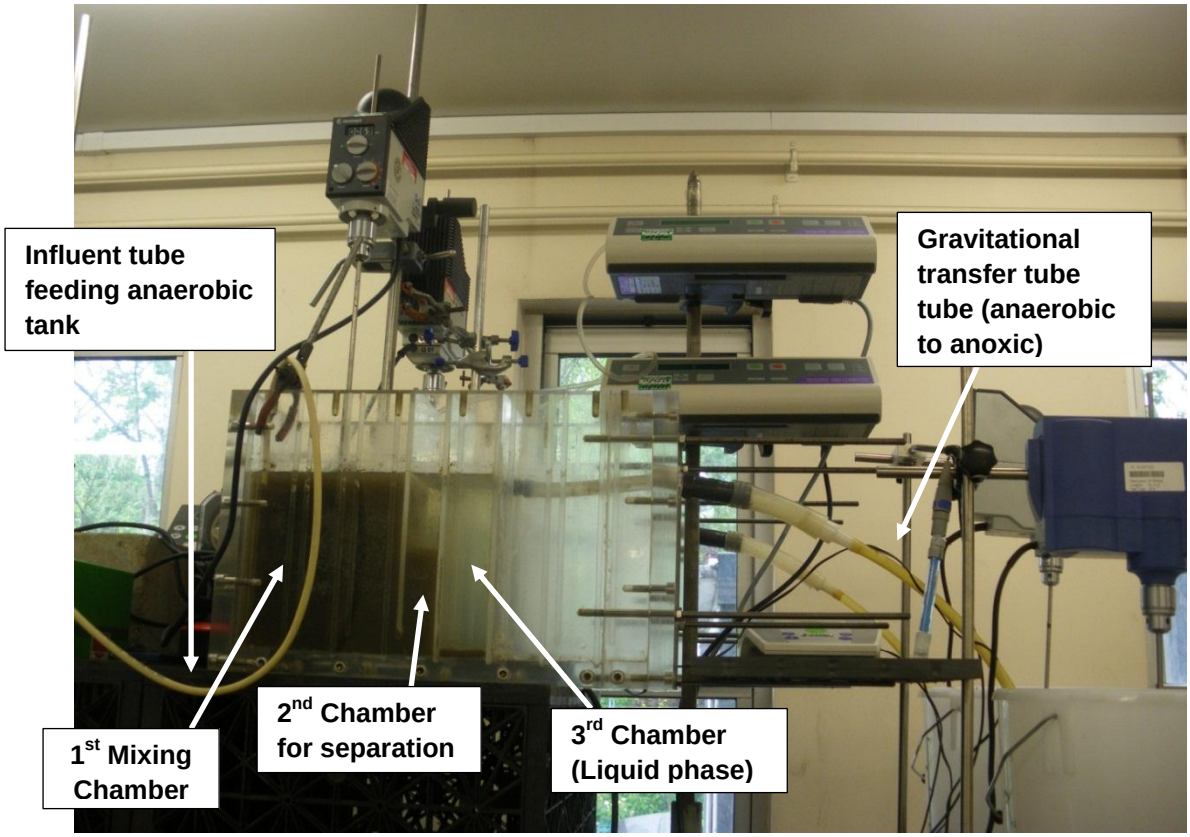


Figure 18: Anaerobic tank.



4.4.2 Anaerobic tank.

The anaerobic tank was fed in influent wastewater by peristaltic pump. Walls were used to separate the reactor into 3 chambers that were interconnected by underflow weir walls.

4.4.2.1 The first chamber

The first chamber was the largest and received discharge of fresh wastewater every 15 minutes, and the period between feeds was used for the calcium chloride to mix the extra-carbon food either milk or sugar complex. The anaerobic tank had 2 main purposes, improving the fermentation process and reducing the MLSS, and thus the TOC, by lowering the growth rate.

- The fermentation process: The first chamber was design to keep a sufficient HRT to allow a proper reduction of complex food carbon by anaerobic (anoxic) heterotrophic pathways. It was hoping to generate higher soluble substrates to microorganisms (S/M) ratio at the end of the anaerobic process.
- The reduction of MLSS was made mechanically, because in order to better reduce the exchange of material between the anaerobic tank and anoxic tank, the flow path was formed to be easier for liquid than the solids:
    1. The MLSS exchange was made from the first to the second chamber into the bottom forcing flows to go upward separating solids from liquid by gravitation.
    2. The stirrer (in first chamber) and the small opening between the chambers one and two, slow speed differences generated turbulence between chambers (first being faster).
    3. The stirrer was set to create sufficient force to keep the sludge in suspension. However the suspension of the sludge was limited to the lower part of the second chamber, improving a gravitational liquid-solids separation.

The stirring speed had to be controlled to avoid MLSS aeration, thus this was achieved with large paddles distributed at different heights on the rod stirrer.

4.4.2.2. The second chamber

The following chamber was provided with an overflow weir, was unstirred and served as a buffer in the way that the solids were retained to allow only liquid rich in assimilable substrates to pass. The limited motion enabled the maximum solids to be retained within this second chamber. The amount of the sludge that slowly builds was related to the MLSS content retained in the first chamber.

4.4.2.3. The third chamber

The third chamber contained the fermented liquid phase that was then discharged into the anoxic tank through a pipe by gravitation. The third chamber would eventually receive MLSS from the second chamber, and this was the signal that a general reduction of MLSS was necessary.

The second and the third chambers HRTs were 2 hours in total, and the first chamber between 3 hours and 4 hours. Keeping most of the sludge in the anaerobic tank also reduced the problems of pipe obstructions and membrane clogging, as well as providing a maximum of liquid rich in VFAs without extra hard COD that could not eventually be biodegraded. There was no exchange of sludge or liquid from the anoxic or aerobic tank into the anaerobic tank (the total capacity was 10 litres) (Figure 16 and 18).

4.4.3. Anoxic tank

Intermittent stirring mixed the anoxic tank to ensure contact between the nutrient from the anaerobic tank, and the MLSS recycle coming from the aerobic tank. The tank was a cylinder with a maximum capacity of 25 litres provided with 4 exit connections given retention times based on 5 litres of capacity, starting at the minimum of 10 litres. In accordance with the desired HRT the MLSS from the anoxic tank discharged into the top of the aerobic tank. The mixing was gently done with three paddles at different levels. The presence of VFA and nitrogen created the anoxic conditions for OHO to denitrify, and for DPAO to remove P and N (Puig *et al.,* 2007).

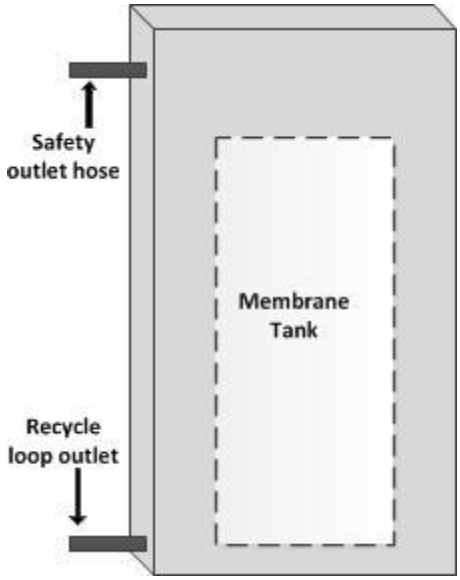
4.4.4. Aeration tank

The aerated bioreactor tank has been made of translucent Plexiglas® to allow visual inspection of the membrane (aeration bubbles, cake building etc.), and sludge flocs behaviour in the tank. The dimension of the internal tank had a maximum of less than 13 litres volume capacity (H: 50cm x L: 35cm x W: 7cm) to hold 10 litres of MLSS, and the 0.5 litre volumetric space occupied by the membrane chamber.

The aeration tank was equipped with two exit openings (Figure 19):

- The first one at the top was used as a reserve allowing up to 10% of extra MLSS capacity as a surge;
- The second one from the bottom was used for the recycle loop to the anoxic tank using a peristaltic pump (Figure 16 and 17).

Figure 19: Aeration tank



Any extra-carbon and calcium came respectively through different hoses at the top using peristaltic pumps (Figure 17, 18). The initial MLSS seed was activated sludge from local wastewater treatment plant.

4.4.5. The membrane chamber

The configuration was based on those common at the industrial sites, membranes placed side by side with a narrow inter-space between them in a chamber so called “the cassette” which are also placed close one to each other. An aeration system is placed below the cassettes, to bubble air and shear physically the walls of the membranes with air escaping through the membranes into the inter-space to the top. It was necessary to mimic the full-scale treatment plant cassette that used immersed membrane Kubota® filtration in aerated tank to keep the performance optimal. Thus to do this, a scaled down purpose built tank was made.

The chamber was designed translucent to observe and assess the clearing capability of the air to maintain the membrane cartridge using air bubbles that channelled inside the cassette and across the membrane walls (Figure 17 and 19). The air was provided by an external compressor, and the dissolved oxygen was always kept to a minimum of 2 mg/L in the aerobic zone. The oxygen level was not a key experimental variable for nitrification and was checked daily manually to ensure if sufficient aeration was provided to the system.

It was originally intended to use the flat sheet membrane used by Nestle (Dalston) at its full scale dairy plant (Zenon ZeeWeed® Ultra-Filtration Type 1000) but reconstructing and particularly welding the reduced membrane was impossible. Therefore another commercial membrane was identified and investigated as being capable handling the required daily flow rates. Figure 20 shows the cartridge details with the following characteristics: the flat sheet Kubota® 'Type 203' has a nominal pore size of 0.4μm. The sheets are made from chlorinated polyethylene and are ultrasonically-welded on both surfaces of membrane panel. The affective filtration area is 0.14 m^2 per cartridge, the external perimeter is 226mm width, 316mm height and 6mm thick.

Figure 21 shows the actual cartridge membrane in the chamber designed for the laboratory usage, and figure 22 provides detailed front and side view of the membrane in its chamber.

Figure 20: Kubota® Type 203

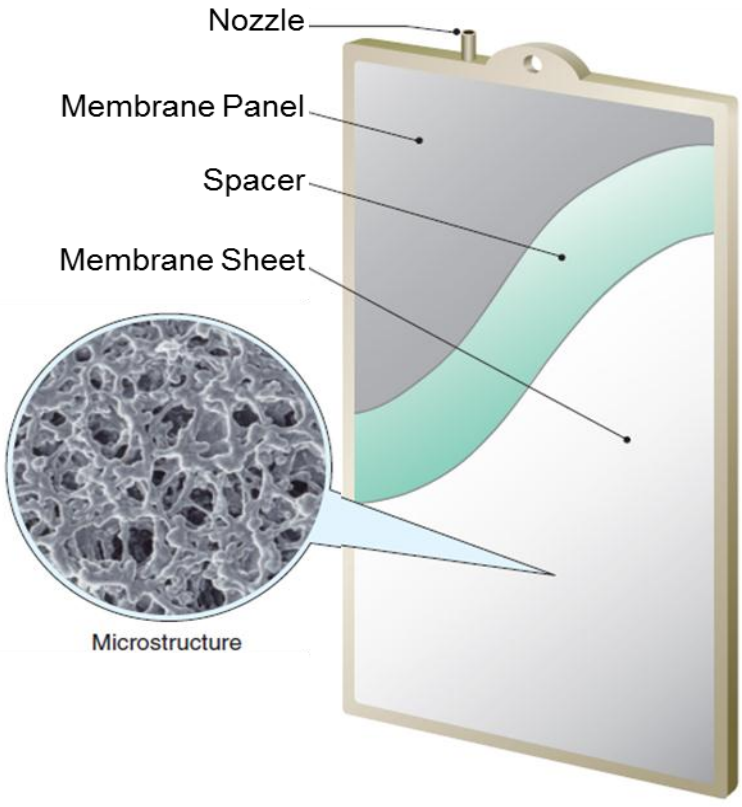
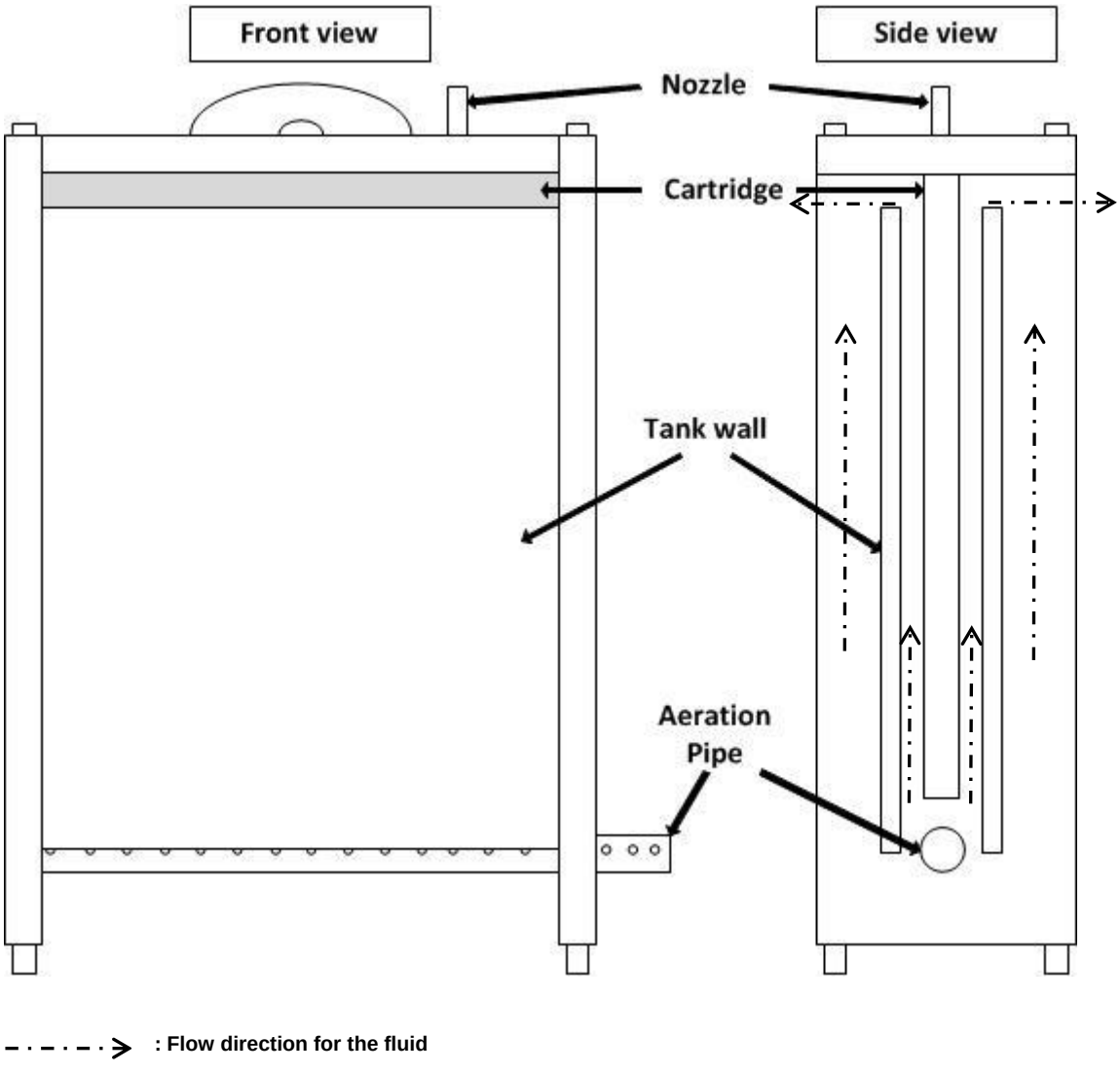


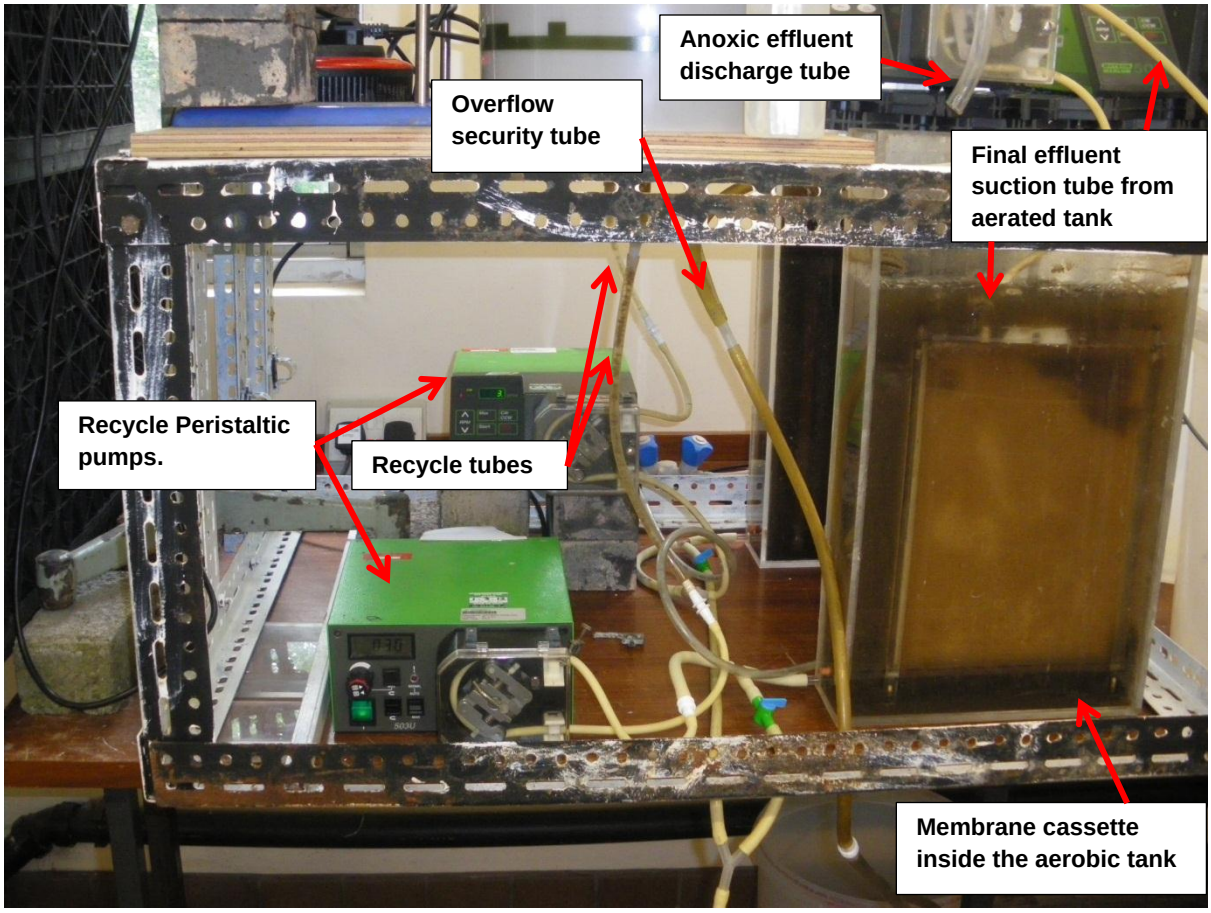
Figure 21: Membrane cartridge in its laboratory cassette.

Figure 22: Views of membrane laboratory cassette.



The aeration pipe was between the two cassette walls that were however shorter than the chamber height to allow an easy entry for the MLSS at the bottom and an easy exit at the top for both, air and MLSS (Figure 21). The air provided circulation, initially checked by dye observations, but also by the ability to keep all the solids in suspension and an appropriate level of aeration for the microorganisms in the tank. The aeration tank connections are shown in Figure 23 below.

Figure 23: Membrane chamber in the aerobic tank



The choice of membrane system was used in order to reduce the footprint of the laboratory, rig and avoid the settling system as bulking was anticipated. It also mimicked the full scale dairy plant wastewater treatment plant under investigation. The retention times in the stages were based on those found in the literature for municipal wastewater using the UCT, EBPR process. In the subsequent laboratory experiments the HRT was adapted, according to the results, to suit the stronger influent.

Selected HRT were:

- Anaerobic HRT between 1.0 – 2.0 h
- Anoxic HRT between 2.0 – 4.0 h
- Aerobic HRT between 4.0 – 12.0 h.

(Coats *et al.*, 2011; Wang *et al.,* 2013)

The study made by Wang *et al.* (2013), assessed the relationship between HRT and biological nutrient removal (BNR) efficiency and concluded that the HRT for COD, TP, TN, $NH4^{+}$-N and KN removal efficiency was optimal at a total of 19 hours for the whole UCT process.

The suction in the membrane, to withdraw the liquid from the MLSS, was done by a peristaltic pump attached to the nozzle on top of the membrane cartridge. The pump was working 15 minutes per hour at slow speed to obtain a flow rate of 10 litres per day. The trans-membrane flux was simply monitored by the amount of permeate collected in the in the final effluent collector tank. As the peristaltic pump was set at constant speed, if the filtrate collected was less than 10 litres in the final tank, the level of MLSS in the aerated tank would increase and reach the security level, at this point the membrane was considered as clogged. A system of membrane rotation was then implemented, the clogged membrane was removed and put into soaking to be cleaned, and replaced by the stand-by unit (kept until needed in the cleaning solutions).

4.4.6 Membrane cleaning

4.4.6.1 Cleaning from Kubota®

Following the Kubota® guidance the cleaning was done with oxalic acid for inorganic precipitates and bleach for organic waste at typical 0.5% hypochlorite concentration. Cleaning operations are advised by Kubota® to be done every three to six months with soaking of 1 hour.

4.4.6.2 Cleaning from our experiments

The appearance of air bubbles in transparent suction hose when pumping the liquid from the membrane was an easy way to monitor the membrane condition. The volume of final effluent would also be lower than expected. Using these criteria, the cleaning process was engaged more often than the 3 – 6 months suggested by Kubota®. In our apparatus the cleaning was done ex-situ whereas in place cleaning is normal at full scale. The main reasons to that were the unknown effect and the possibility to harm the stable culture in the aerobic tank during chemical reagent action.

Our cleaning was ex-situ with the following sequences to clean the membranes:

1. Soaking in 0.5 % bleach for 4 hours;
2. Soaking in 1.0 % in acetic acid for 2 hours;
3. Connection to a peristaltic pump for the circulation of water-acid mix for 10 to 20 minutes;
4. Cleaning by recirculation in clean tap water for 15 minutes.

4.4.6.3 Conclusion

The conclusion from our experiments was that the two chemical cleaning stages were necessary but the oxidation followed by acid sequence was more effective than the reverse to give the best membrane cleaning. Tests after cleaning were made using the same peristaltic pump setting, to filter 1 litre of tap water which took 2 minutes. The first cleaning treatment usually gave between 600 – 800 ml effluent and the second treatment provided at least the 1 litre expected, if not the cleaning process was repeated until it did.

4.4.6 The nucleation: Final Effluent Second Treatment (F.E.II) process.

An additional step for nucleation was added for the reduction of soluble P within the final effluent. Through this process, it was expecting that the excess of soluble P would bind to the calcium phosphate as nuclei.

4.4.6.1 Description of nucleation tank

The nucleation tanks were two 1 litre beakers, 1 for each line. The designs in Figure 16 did not include the nucleation tank since this was added in Phase I (Section 6.2) experiments. The process was carried out manually daily as shown in Figure 24. Figure 25 shows a picture made after calcium phosphate precipitation, before the sample collection. A beaker of clean tap water has been included for comparison purpose.

Figure 24: Final effluent treatment process

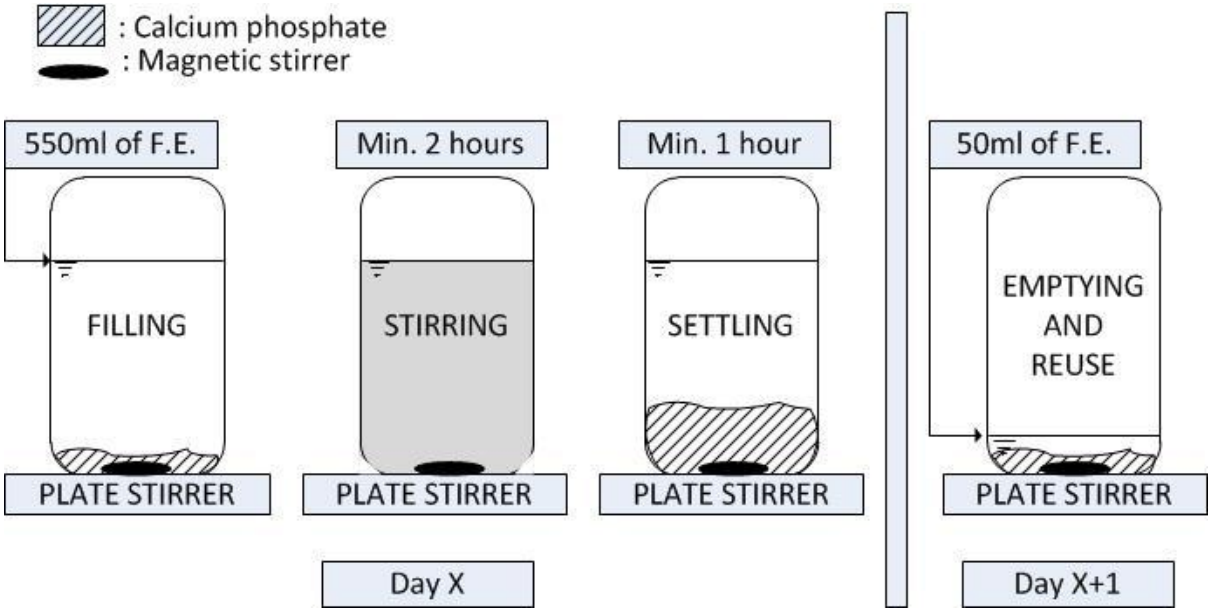
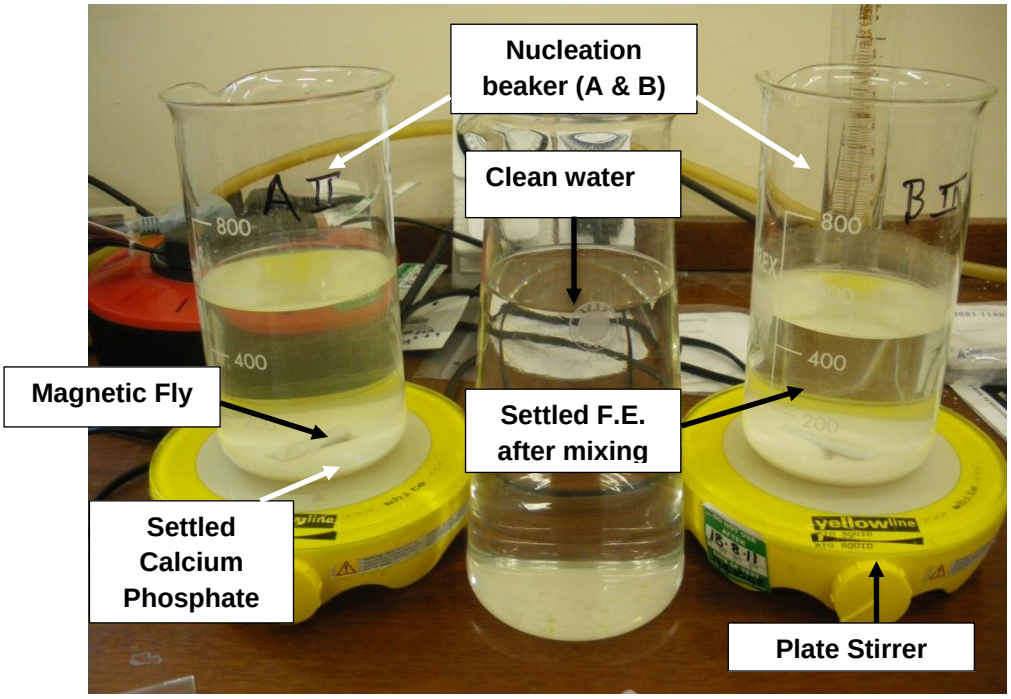


Figure 25: Nucleation laboratory rig



4.4.6.2 Final effluent treatment methodology

For the start up a 1 litre beaker was placed on a magnetic plate stirrer and 3g of calcium phosphate ($Ca_3(PO_4)_2$) added. 550ml of F.E. sample was added to the calcium phosphate in the beaker (i.e., referred as F.E.II), and content was gently and homogenously mixed for 120 minutes with an intermediate precipitation after 60 minutes to allow the calcium phosphate to settle.

20 ml of supernatant was collected with a syringe, filtered through a 0.45μm pore size filter and stored in a sealable 15 ml conical sterile polypropylene tube. It was stabilised with 2% content concentration nitric acid. The sample was placed in the cold room to be analysed with a maximum of 3 days.

The following day ± 500 ml of supernatant was withdrawn from the beaker and replaced with ± 500 ml of fresh F.E. to repeat the process daily. The calcium phosphate precipitate was thus reused until another phase of experiments. This post F.E. nucleation process was hypothesised to create larger molecules to assist P precipitation (Literature review section 2.6.7.4).

There was another calcium addition of $CaCl_2$ of 200 ml at 250 mM concentration into the anaerobic and/or aerobic tanks equivalent to 2000 mg Ca^{++}. The reasons hypothesised to balance the dosage of $CaCl_2$ were:

- This injection of calcium purpose was to mimic the effect of Ca in milk independently of the proteins. The idea was to fix excess of soluble phosphorus that could not be absorbed by PAOs, whilst avoiding a side effect of chelating too much of the available soluble P that could disturb their metabolism reducing the biological accumulation of phosphorus (Barat *et al.,* 2006, 2008, 2011).

- Second, as it was mentioned in the literature, the competitive nucleation processes are very important (Mekmene *et al.,* 2009; Barat *et al.,* 2011; Habraken *et al.,* 2013) and most studies used synthetic wastewaters with the calcium and the phosphate precisely measured and controlled (based on precise thermodynamic behaviour calculations) to avoid this. In this study it was concluded that milk additions would not allow for these precise calculation, and thus Ca^{++} would be provided in excess.

- Thirdly, this study attempt through sequential increase of soluble phosphorus to approach the upper limit of chelating PO_4-P by determining a suitable milk concentration as a AOM. Therefore, the research could provide data for a constant removal of P that could be achieved in more practical and applied conditions.

Pouliot *et al.* (1991) research was one of the main references for nucleation using a precursor seeding. This study used the tribasic calcium phosphate ($Ca_5HO_{13}P_3$), with calcium content between 34.0 and 40.0 % (Aldrich Chemistry) as the nuclei.

Due to their complex chemistry and small dimensions, unravelling the structural details of the calcium phosphate clusters in their native hydrated state, or the mechanism by which they aggregate, was not attempted but recommended as a separate project. The method of analysis is very complex and the results would have been poorly reproducible given the scope of this project. There is competitive nucleation in the bulk solution since the ions and complexes formed are not strictly calcium and phosphorus.

4.5. Instrumentation and Method Analysis

4.5.1 Daily operations

1. The pH, the D.O. or the RedOx were manually measured on a daily basis (see the list of instrumentation 4.5.3).
2. Collection and filtration to obtain supernatant pre-filtered for chemical analysis:
    - In the anaerobic tank, 35ml of supernatant was withdrawn from the 3rd chamber;
    - In the anoxic tank, 35ml of MLSS was withdrawn;
    - In the aerobic tank, 35ml of MLSS was withdrawn;
    - These 3 samples were placed in the machine for centrifugation during 10 minutes at 9.000 rpm to speed the settling with g force;
    - Final effluent, 35ml was withdrawn from the tank;
    - Therefore each of the 4 supernatants was initially filtered with an 11 μm pore size filter paper.
    - 20ml were then subsampled with a syringe to be filtered again through a 0.45μm pore size filter. The filtrate was injected in a sealable 15ml conical sterile polypropylene tube and stabilised with 2% content concentration of nitric acid.
3. The samples were placed in the cold room for a maximum of 2 days (4°C) for 2 days maximum before analysis using the ICP.
4. The final effluent was filtered again (0.45 μm pore size filter) without centrifugation.
5. The nucleation tank supernatant was collected with a syringe after at least 1 hour of settlement with the $CaPO_4$ and filtered with a filter 0.45 μm pore size.
6. The extra carbon sources (milk and sugar complex) were prepared every day fresh to avoid curd formation with milk or mould with sugar (exception made for the week-end).
7. The NTU for the final effluent was measured daily only for the final effluent.
8. The TOC was measured for the anaerobic, anoxic, aerobic supernatants and the final effluent.

4.5.2 Weekly operations.

1. The total suspended solids and the volatile suspended solids were measured every week. The starting inoculums were analysed and the MLSS was also quantified.
2. 1 litre of 0.5M $CaCl_2$ was freshly prepared to be diluted and put in the chemical dosing tank.
3. Collection of 120 litres of settled sewage in Loughborough wastewater treatment plant and placed in cold room at 4°C. 50 litres were withdrawn and enriched with PO_4-P every 2 days.

4.5.3 Instrumentation analysis.

4.5.3.1 Inductively Coupled Plasma (ICP – OES)

Initially the Thermo Jarrell Ash Atomscan 16 was used until to measure the P concentration in sample until the 11/11/2011, when unfortunately the machine failed. Therefore, a new machine has been used, the ICP-OES (Shimadzu® ICPE-9000), until the end of the research. The analyses were carried out according to 3125 A in the Standard methods (APHA) and always within a week of the samples being taken. The precision for P analysis is not noted in the manual.

Water samples preservation and preparation

Initially, water samples were analysed without dilutions, and if concentrations were found to be above the highest calibration standards, subsequent dilutions were required and appropriately documented.

1. A first preparation method of water samples measured the P content in samples after acid digestion.
    a. The samples were collected without prior filtration
    b. Samples were mixed with a combination of 2 ml hydrochloric acid and 3ml nitric acid (Aqua Regia),
    c. For digestion, mixes were placed in tubes for a microwave digestion (Mars Xpress – CEM).
    d. After digestion, filtrate using (11 µm filter pore size), samples were analysed with the ICP (Sequential Plasma Emission Spectrophotometer Thermo Jarrell Ash Atomscan 16, serial no. 1846, model TJA).

2. A second method was used to improve the samples analysis and thus changed the sample collection, preservation and preparation techniques as the first method overestimated the phosphorus content.
    a. Before ICP-OES (and IC) analysis, the water sample was filtered to less than 0.45 µm and preserved by cooling to maximum 4°C within 4 hours.
    b. The samples were acidified using nitric acid HNO_3 at 2% volume concentration at least 16 hours before ICP (IP) analysis.
    c. Samples were analysed with the ICP, firstly using the Themo Jarrell then the Shimadzu

ICP-OES Procedures

Prior to sample analysis the detection limit was set following the preparation of solutions for calibration:

- Reverse Osmosis ultra-pure water filtration was used to rinse the tube, probe during warm up.
- A calibration blank and calibration standards for the:
    - First method :RO water as blank and calibration standards for a 5 point calibration (20mg/L – 40mg/L – 60mg/L – 80mg/L – 100mg/L) (see section 5.4 for more details)
    - Second method: RO water with 2% HNO_3 as blank, and calibration standards for a 9 point calibration 0.25mg/L – 0.50mg/L – 1.0mg/L – 2.5mg/L – 5.0mg/L – 10.0mg/L – 25.0mg/L – 50.0mg/L – 100.0mg/L (all diluted in RO water with 2% HNO_3).
- A check blank solution and verification solutions made from stock solutions independent of the calibration standard stock solution.
- Samples were analysed from the weakest P concentration to the strongest (final effluent after nucleation to anaerobic).
    - A blank solution was inserted every 5 analyses for control and cleaning. The results were always below the Instrument Detection Limit (IDL) related to the ICP
    - Solutions independent of the calibration standard stock solution followed the blank solution and the specific limits were for both ICPs between 95 – 105% immediately after calibration (for the first 10 analyses), and between 90 – 110% thereafter

The calibration was considered good only if the calibration curve had the correlation coefficient (R^2) above 99.0%.

The detection limit at P atomic wavelengths using:

1. Thermo Jarrell Atomic Scan 16: limit of detection 0.060 ppm (177.499 nm)
2. Shimadzu ICPE 9000: Between 1 and 10 particle per billion (ppb) (Shimadzu manual) (177.499 nm and 213.618 nm)

4.5.3.2 Ion chromatograph (IC)

A Dionex DX100 Ion Chromatograph (IC) (Dionex-100, column type AS 9-HC, eluent 9 mM Na_2CO_3, flow rate = 1.0 ml/min and injection volume = 25 µL) was used to measure the soluble NO_3 (Nitrate) level in the experimental samples. The procedure in APHA 4110 C was followed, SD is reported to be 2.0 mg/L for raw waters. Conductivity detection of inorganic strong acid anions, such as nitrates and sulphates, was reported as accurate to 1 mg/L in the operating manual (Dionex 1998).

4.5.3.3 Total organic carbon analyser (TOC)

Total organic carbon was analysed by high temperature catalytic combustion method (APHA 5310 B) using a Rosemont-Dohrmann DC-190 total carbon analyser (injection volume = 20 µL and 2 stage filtered).Precision for filtered samples is reported as better than 5% in the APHA manual.

4.5.3.4 Chemical Oxygen Demands (COD)

COD was analysed using the closed reflux colorimetric technique as described by Standard Methods (APHA,5220 D 1995). Precision is reported to be +/- 5%. The chemical oxygen demand was determined using a Hach heater model 45600 COD Reactor testing the cells at 2.000 and 20.000 concentrations. The results were read using the bench-top Palintest Photometer 8000.

4.5.3.5 Turbidity

Sample turbidity was measured using APHA method 2130 B using the Hach 2100N Turbidimeter. Appoximately 30 ml sample was poured into specific glass for turbidimeter readings. The samples were read 3 times to provide an average reading. The calibration was made once a week using the standard solutions provided. Precision is reported to be +/- 2.5% in this range.

4.5.3.6 RedOx Conductivity

RedOx was measured using the Mettler Toledo Seven Easy with the model probe InLab Redox Ag every day, and the calibration was made once a week. Method 2580 B (APHA) was used precision is reported to be +/- 10mV but in clean water only.

4.5.3.7 Dissolved Oxygen (DO)

DO was measured daily using a portable apparatus (Yellow Springs Model S4) and was calibrated weekly. Method 4500-0 G (APHA) was used and precision reported as +/- 0.5mg/L.

4.5.3.8 pH

The pH was measured every day using APHA 4500-H^+ B (Hanna Instruments HI 9812.) No artificial adjustments were made during the treatment experiments. The calibration was made once a week and precision expected to be better than +/- 0.1 pH (APHA).

4.5.3.9 Mixed Liquor Suspended Solids / Volatile Suspended Solids

Total suspended solids (TSS) and volatile suspended solids (VSS) were determined according to APHA Standard Methods (1995) method 2540 B from unfiltered samples. The MLSS assessment was made once a week, usually after the week-end. 50ml samples were used to assess the dry sludge solids(water-bath then oven at 105^{o}C) and the ashes of this sludge (VSS) following furnace treatment at 550^{o}C). The samples were cooled in a desiccator before weighings. Precision is reported to be 5% for the TSS in the manual but is not specified for VSS.

4.5.3.10 Other

Other equipment used during the experiments is listed below:

- Drying Oven (120°C): ELE International
- Furnace: High Temperature Chamber Furnace AAF1100 (AAF11/7) - Carbolite
- Precision Balance:
    - Precision Balance AC 211S - Sartorius
    - Precision Balance HM-202 – A&D Weighing
- Peristaltic Pumps: WATSON MARLOW (Models 503 U/ 505 S/ 505 DU)

- Overhead Stirrer:
    - 1: HEIDOLPH Models RZR 2021/ 2041/ 2102/ 2051.
    - 2: EUROSTAR, IKA – WERKE.
- Plate-stirrers:
    - 1: IKA – BIGSQUID (Yellow Line)
    - 2: IKA – COLOR SQUID
- Hot Plate Stirrer: STUART, Model SB 302/ CB 302
- Centrifugation: Eppendorf, Bench-top digital Centrifuge 5804
- Microwave Digester: Mars Xpress – CEM

- COD Analyser: PALINTEST, Model 8000,
- COD tube test: PALINTEST, PL454 (COD/2000); PL456 (COD20000)
- TOC syringe: SGE, Model 100 R-LC (R08-T6342)
- Filter qualitative papers 11μm: WHATMAN (Cat N°1001 125), 125mm ø x 100.
- Syringe Filter 0.22μm, 0.25μm (PVDF membrane): RESTEK
- Micro-filters 0.45μm (PTFE membrane): Puradisc – WHATMAN
- Disposable Plastic Syringe: 10ml, 20ml, 50ml – Fisherbrand - Fisher Scientific.
- Beakers, Measuring cylinders, high precision micro-pipettes (100–1000μl), tongs, ceramic dishes.
- Hot Water Bath: NIKEL ELECTROD Ltd, Serial N° 66746
- Etc. …

4.5.3.11 Chemicals and products used

- Acids: Fisher Chemical
- Calcium chloride dihydrate: SIGMA-ALDRICH
- Calcium phosphate: SIGMA-ALDRICH
- Glucose, starch, dextrin: SIGMA-ALDRICH
- Cleaning agent for reusable tubes, funnels and others: Neutracon from DECON

4.5.3.12 Research experiments

After the laboratory trials (Section 5.0) the research tested several parameters where the experiments isolated and compared them one to another. Table 19 summarises the experimental steps and experiments that were carried out during the research.

Table 19: Summary of the steady state continuous experiments (years 2 and 3)

Control Line	Time (Weeks)	Feed Charact.	Chemical Added/24h Concentration	Zone/Volume	**Test Line**	Time (Weeks)	Feed Charact	Chemical Added/24h concentration	Zone/Volume
MILK CONCENTRATION ASSESSMENT									
PHASE I: 6 WEEKS.									
Period 1	**3**	**- WW SS - Glucose**	**200ml $CaCl_2$ (250mM)**	**-Anaer/100ml -Aerob/100ml**	**Period 1**	**3**	**- WW SS - 1.0% Milk**	**200ml $CaCl_2$ (250mM)**	**- Anaer/100ml - Aerob/100ml**
Period 2	**3**	**- WW SS - Glucose**	**200ml $CaCl_2$ (250mM)**	**-Anaer/100ml -Aerob/100ml**	**Period 2**	**3**	**- WW SS - 2.5% Milk**	**200ml $CaCl_2$ (250mM)**	**- Anaer/100ml - Aerob/100ml**
ZONE CHEMICAL DOSING ASSESSMENT									
PHASE II: 3 WEEKS.									
	3	**- WW SS - 1 % Milk**	**200ml $CaCl_2$ (250mM)**	**-Anaer/ 0ml -Aerob/200ml**	**Parallel running Experim.**	**3**	**- WW SS - 1 % Milk**	**200ml $CaCl_2$ (250mM)**	**- Anaer/200ml - Aerob/ 0ml**
CARBON SOURCE EVALUATION WITH FINAL CONFIGURATION RUNNING									
PHASE III: 8 WEEKS.									
Period 1	**4**	**- WW SS - P high conc.**	**200ml $CaCl_2$ (250mM)**	**-Anaer/100ml -Aerob/100ml**	**Period 1**	**4**	**- WW SS - Glucose Mix - P high conc.**	**200ml $CaCl_2$ (250mM)**	**- Anaer/100ml - Aerob/100ml**
Period 2	**4**	**- WW SS - P high conc.**	**200ml $CaCl_2$ (250mM)**	**-Anaer/100ml -Aerob/100ml**	**Period 2**	**4**	**- WW SS - 1% Milk - P high conc.**	**200ml $CaCl_2$ (250mM)**	**- Anaer/100ml - Aerob/100ml**
RUNNING FINAL CONFIGURATION FOR 8 WEEKS.									
PHASE IV: 8 WEEKS *(Only a single line was used)*									
Period 1	**1**	**- WW SS - 1 % Milk - Natural P conc.**	**200ml $CaCl_2$ (250mM)**	**-Anaer/100ml -Aerob/100ml**					
Period 2	**4**	**- WW SS - 1 % Milk - P high conc.**	**200ml $CaCl_2$ (250mM)**	**-Anaer/100ml -Aerob/100ml**					
Period 3	**3**	**- WW SS - 0.5 % Milk - P high conc.**	**200ml $CaCl_2$ (250mM)**	**-Anaer/100ml -Aerob/100ml**					

5.0 Laboratory Trials.

5.1 Introduction.

A study of P removal at a full scale dairy plant was the initial stimulus for this project, but to increase the academic value of the research, it was obvious that there were advantages in the laboratory environment to carry out experiments, where it was possible to control the conditions. This control was very valuable as the biological process is dependent to its surroundings. The other valuable aspect was to improve the experimental reproducibility, and being able to collect and measure the data at frequent intervals during the day.

External companies to either build the laboratory reactor or for technical support (i.e., external data analysis, engineering assistance) were not available to the research and the system was bespoke. Kubota® provided the new membranes for the membrane bioreactors.

The laboratory model activated sludge plant was built from scratch and modified during the experimental programme to suit the experimental conditions. Thus the aim was to design simple and well controlled experiments to be at least duplicated and therefore, usually the tests lasted at least 3 weeks.

5.2 Batch Tests Trial:

5.2.1 Introduction.

Most previous academic research refers to laboratory scale sequencing batch reactor tests (Lopez-Vasquez *et al., 2009*). The idea was to start experiments with sequencing batch reactors with the main advantage of being able to compare with previous work. Batch tests are easy to control and reduce uncertainties related to large scale complicated machinery.

5.2.2 Material and methods.

Initially four 1 litre beakers were divided into 2 process trains with each beaker on an individual magnetic plate stirrer. Each process train had one anaerobic beaker and one aerobic.

Each experiment was designed to last a week to investigate the influence of different quantities of milk addition and on P removal. The tests were carried out at room temperature.

5.2.3 Experimentation.

The 25 litres of settled sewage collected from the WWTW were stored and aerated with gentle stirring in the cold room (4°C). Soluble P after simple filtration (11μm pore size), nitrate, pH, TOC, the SOUR and the suspended solids were measured before starting the experiments.

4 litres of wastewater were taken at a time from the feedstock and pre-treated (i.e., raised to room temperature, aerated with gentle stirring or deprived of aeration for the anaerobic conditions).

The experiment consisted of two lines as follows:

- Line 1: milk addition in either anaerobic or aerobic beakers.

- Line 2: used as control and fed with sugar mix.

After 3 hours of contact time, tests analysis were done, after simple filtration of 11μm pore size, on the aerobic samples withdrawn from the beakers for SOUR testing (D.O. in the aerobic stages were kept at minimum 3 mg of O_2), redox on anaerobic samples and soluble P level measurement. After another 3 hours following the first tests, the analysis was repeated on the same MLSSs to measure the changes.

5.2.4 Results.

Practical problems confounded the results from the beginning, namely the aeration caused foaming and MLSS losses because of the protein in the milk. Neither the control anaerobic nor test anaerobic beaker reactors reached a low redox (never less than - 50 mV), thus the anaerobic conditions were never achieved, making P release difficult. Therefore, most of the data collected were inconclusive for interpretation but also experiences conditions were difficult to reproduce using this simple system as test methods. These preliminary tests nevertheless suggested that a continuous flow system (anaerobic-anoxic-aerobic) was needed to get reasonable results. The main reasons were: to keep the continuity of the acclimatized microorganisms, have a larger amount of MLSS which would not be disturbed by foaming, provide sufficient volume for sample collection and allow better oxygen transfer through the surface area.

5.3 First period membrane bioreactors trials:

5.3.1 Introduction.

The literature and scoping study established that the use of a membrane bioreactor would increase the reliability and reduce the footprint of the rig. The use of the membrane provided an easy way to control the hydraulic retention time (HRT) and the sludge retention time (SRT) through permeate flow rate.

This group of experiments were focused on the release and uptake of soluble P with a continuous flow in the standard BPR process flow sheet of anaerobic-anoxic- aerobic. A common variation of the standard BPR process sequence is an additional recycle of aerobic mixed liquor to the anoxic zone to reduce the nitrate load to the anaerobic zone to ensure anaerobic redox is achieved. A membrane plant however operates at 3-4 times the MLSS concentration and there is a possibility that recycling at these concentrations could also release extra VFA from sludge breakdown interfering with the need for the PAO to produce polyphosphate. Therefore this additional recycle loop was not used in these first experiments to provide a control period to study the impact of milk alone.

Every day after data collection, sludge was returned manually from aerobic tank to anoxic tank, because of this slow transfer of MLSS there was a gradual emptying of the anoxic tank. The design for the anoxic tank was not very clear at this stage.It must be reminded that the anaerobic tank had an original design of 3 interconnected sub-chambers (See sub-section 4.4.1.2) and that at the end of the anaerobic process only the enriched liquid phase entered to the anoxic tank. The suspended solids were almost totally confined and mixed in the first anaerobic chamber.

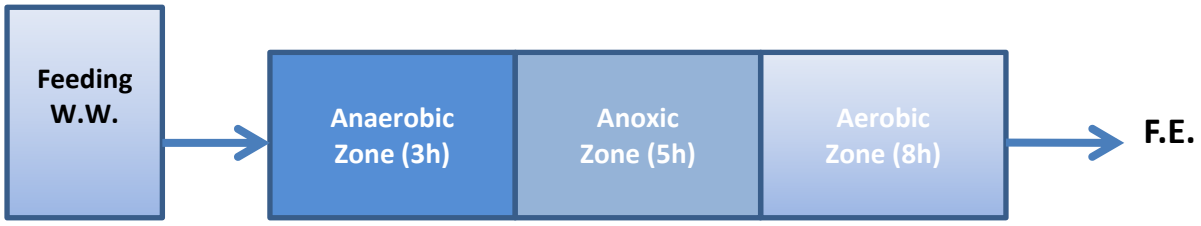
5.3.2 Material and methods

The membrane bioreactor was an experimental membrane bioreactor (MBR) set up using a Zenon Zeeweed® Membrane with a pore size of 0.2 μm. This was a full scale membrane originally 1.2 m height which was too big for a bench test, so this was reduced to 35 cm by cutting and removing a large portion (as noted in the methods).

The principle was the simple version of the University of Cape-Town design (UCT) which used gravity from the first tank through to the final aerobic tank. The system was designed in a 3-5-8 hour HRT scheme for respectively anaerobic-anoxic-aerobic stages (Figure 26). Two peristaltic pumps were used, one to suck the permeate from the aerobic tank through the membrane, the other pump to feed wastewater influent tank to the anaerobic tank.

This first test period used this membrane that was carried out for 8 weeks (16/08/2010 to 14/10/2010) with settled wastewater from the local wastewater treatment works.

Figure 26: Continuous flow with (3-5-8) UCT principle



5.3.3 Results and Discussion.

The first experiment results reported trends on graphs and data collection were made for:

1. Growth of mixed liquor suspended solids (MLSS) in the aeration tank;
2. P concentration in influent, anaerobic tank, anoxic tank and effluent;
3. The influent wastewater characteristics as collected from the local domestic wastewater treatment plant.

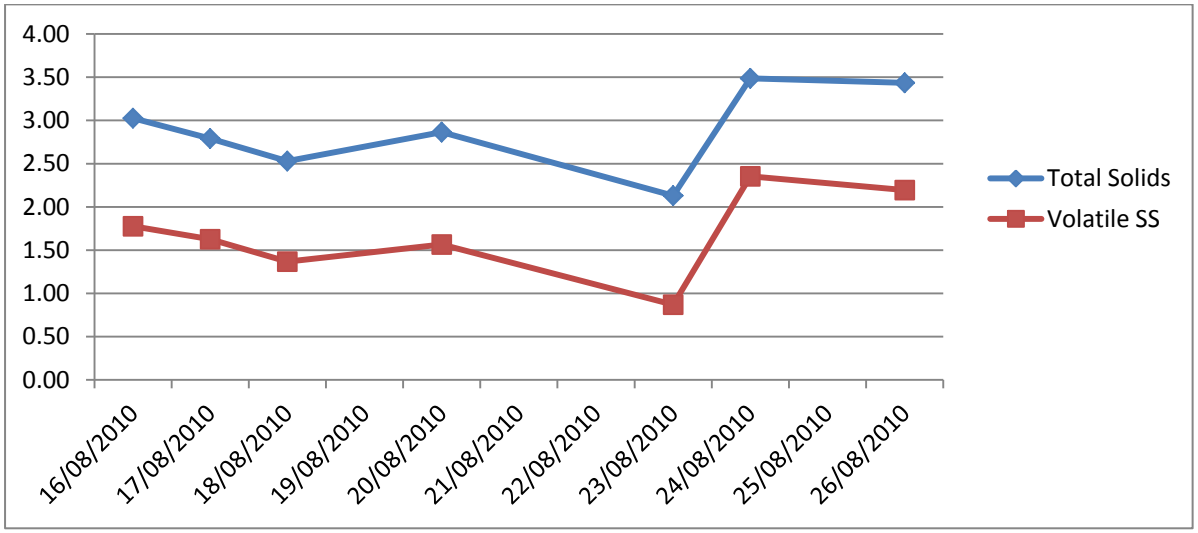
Results from this first period are shown in Figure 27 and Figure 28 below.

5.3.3.1 Evolution of mixed liquor suspended solids (MLSS) in aeration tank.

A first step was to increase and to acclimatize the bacteria to the laboratory conditions and the feed provided. The original biomass inoculum was taken from the aeration tank of the local wastewater treatment plant. Data collection began after a running in period of 15 days (typical sludge age at Loughborough WWTP) to allow time to microbiology for acclimatization and establishment of steady state conditions.

Figure 27 shows unconvincing growth during the first 10 days of tests (following the 15 days of commissioning). The MLSS was higher at the beginning and decreased slowly partly because large aeration bubbles expelled suspended solids and also as a consequence of flocs formed to the walls and base of the tank. These flocs from the walls precipitated and were not easily kept in suspension when they were scraped back into the tank. Possible explanations were that they may have been too dry and then too dense to be re-suspended in the existing mixed liquor. The new growth was not rapid enough to offset this floc precipitation to the bottom and walls of the tank. The concentration of suspended solids in the influent was ignored because the feed tank was not agitated and the feed settled. The final effluent had no measurable suspended solids after membrane filtration and therefore only MLSS figures in the aerobic tank were analysed.

Figure 27: Evolution of MLSS in aeration tank in g/L.

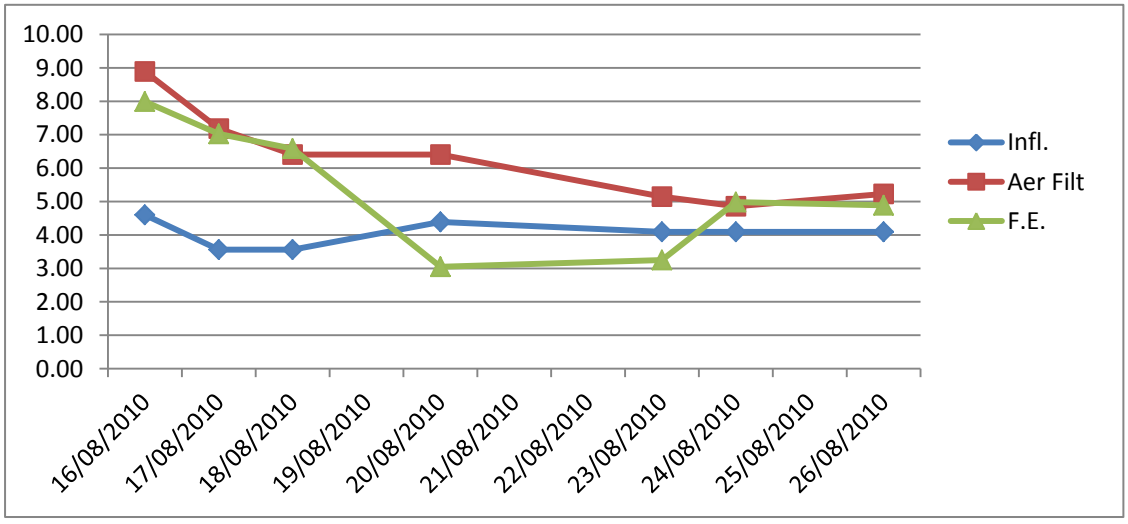


5.3.3.2. Phosphorus concentration in influent, aerobic supernatant and F.E.

The influent wastewater had an approximately constant P concentration of 4 mg/L. Samples were collected from the aerobic zone (Figure 26) after simple filtration (Filter Paper nº1 with an 11 µm pore size) and from F.E. (membrane filtration with a 0.2 µm pore size).

The phosphorus concentration in Figure 28 shows release of P during the process from the MLSS which then reduced in the aeration tank as would be predicted from the literature review. Unfortunately, the discharge in the F.E. was equal to the load, thus there was no overall removal of P. The average final effluent P was around 4 mg/L in the supernatant after simple filtration and was equal to the feed except for two samples. The speciation of the P could be different although soluble since the membrane was not reducing the P content further. The P concentration in the F.E. followed the same pattern as the suspended solids in Figure 27.

Figure 28: P concentration in first trial



5.3.4 Conclusion.

The inconsistency of P measured in the aerobic and final effluent confirmed the variability in performance of the standard BPR as anticipated by the literature reviewed. It was concluded that the micro-organisms needed support to improve the EBPR usually this is achieved by an increase in VFA or addition of coagulant. The experiment showed that the membrane was not capable of removing extra P which must therefore have been soluble. The membrane was efficient at removing all the particles as the final effluent was clear with NTU between [0.1 – 0.8]. For this first part of the experimentation the laboratory modification to the original membrane design provided satisfactory solids separation results. The aeration system however expelled solids to the walls and dried them therefore, a modification to the aeration was necessary to avoid solids losses in the second experiments described in 5.4.

5.4 Second test membrane bioreactors trials.

5.4.1 Introduction

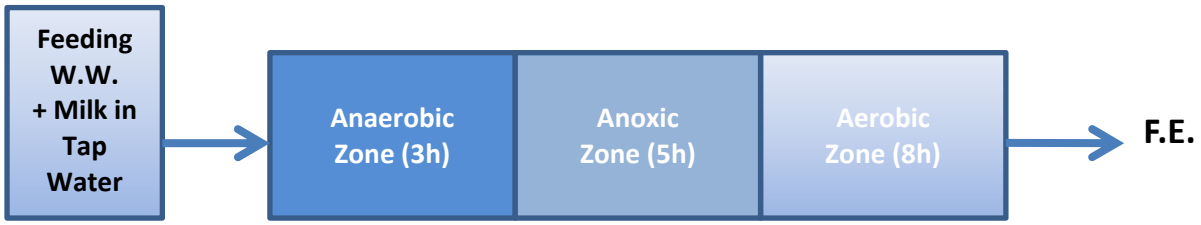
Figures 27 and 28 from the first trial showed that despite a constant load on 4 mg/L of P from influent, the concentration of total P did not reduce overall by sludge accumulation. It was not possible to determine whether this meant no metabolic change or just that release of P anaerobically was equivalent to the re-uptake in the aeration zone. The next experiment was to assess the impact of extra P load together with milk addition as external carbon source to mimic the previous work on additional carbohydrate carbon to improve BPR.

The membrane filtration and zone HRT was the same as the previous experiment (see Figure 29). Addition of phosphate was done into the influent directly using Di-sodium phosphate (Na_2PO_4). The variation of P concentration is shown in Figure 30, from a continuous 3 week test run including the week-ends. Fresh feeds for the weekends were made on Fridays otherwise daily, after collecting data.

The process was carried on with the same biomass culture following the first trial, at slightly more than 4.5 g/L of MLSS in the aerobic tank. The feed was supplemented with 5% milk as a proportion of the domestic wastewater to approximately double the COD concentration provided.

Except for the final effluent, all supernatant samples were obtained after filtration through a filter paper of 11 μm pore size.

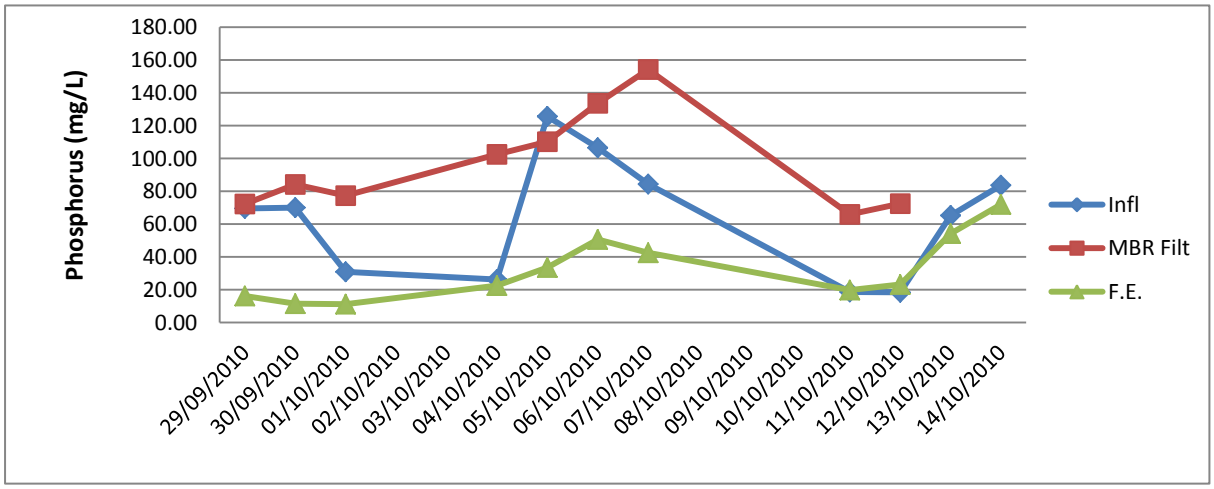
Figure 29: Continuous flow with (3-5-8) UCT principle.



5.4.2 Results and discussion.

Figure 30 describes the curves from the influent, the supernatant from the aerobic tank after filtration, and the final effluent.

Figure 30: P concentration in second trial.



*13/10/10 and 14/10/10: no figures because it was difficult to filter within a reasonable time

The comparative data shows there has been release of P which in this case was not fully taken up in the aerobic zone and followed the same trend as the feed. The aerobic curve did not show a cumulative increase in P and this was interpreted as suggesting that P was reabsorbed within the MLSS but following a lag time in response to a change in the influent P concentration. The relative high P figures in the aerobic zone can be explained partly by the fact that some microorganisms, mostly PAOs, passed through the 11μm filter pore size, and therefore after acid digestion, the figures described the total P from the sample rather than only the soluble P content. Thus in this experiment the membrane usefully removed P.

5.4.3 Conclusion.

The final effluent showed good retention of the P despite the high influent P load. The efficiency was affected by the variability of the feed. Unfortunately the Zenon membrane in these first experiments was cut and challenging to maintain. The seal at the bottom of the membrane leaked and no efficient material was found to solve the problem. Normally the membranes are ultrasonically welded to be firmly attached to the membrane walls. Eventually, the samples were taken directly from the exit tube. Particles were easy to see with the naked eye after membrane filtration, and therefore demonstrated that the membrane had building weaknesses. Therefore it was not possible from this experiment as to whether that the milk could provide the coagulation agents to chelate soluble P. It was concluded therefore that the research needed a new suitable membrane to continue the study.

Despite these solids losses, the biomass increased rather than steady and reached 6 g/L from 4 g/L within the 15 days.

5.5 Batch tests on calcium as coagulant aid.

5.5.1 Introduction.

The purpose of these tests was to assess the possibility of calcium adjuvants to aid the P retention. Iron and aluminium are the most common but lime (calcium oxide - CaO) has also been used for sludge treatment and could be more sustainable for recycling. The reported problems with lime are the poor solubility and effect on pH. Calcium chloride ($CaCl_2$) therefore was therefore tried here. Tests were made with mixtures of calcium as coagulant poured in MLSSs either in the anaerobic or aerobic condition. The idea was to use an assessment trial.

5.5.2 Materials and Methods.

Based on Assobei *et al.* (2004), 2 litres of MLSS were withdrawn from the aerated tank containing an average soluble P of 50 mg/L. Tests using lime (1.4 g/L of CaO, Table 20) was compared to tests using calcium chloride ($CaCl_2$ at 50 mM, in Table 21). All beakers were mixed on a magnetic plate stirrer, but the 2 aerated beakers were equipped with small portable aerating system.

Before starting the experiments, it was necessary to wait for anaerobic conditions for these tests. The addition of chemicals was provided with gentle mixing, and a contact time was programmed for 30 minutes only since the major chemical reactions occur in the first 10 minutes. The analyses were made on filtered samples (11μm filter paper pore size) after acid digestion for the ICP analysis as described in Section 3.

5.5.3 Results and discussion.

Table 20: Test of CaO on sample for each stage

	pH + Chem	Tot P initial	Tot P final
Anaerobic 1 (pH: 7.0)	11.6	47.22	24.75
Anaerobic 2 (pH: 7.1)	11.5	54.13	27.27
Aerobic 1 (pH: 7.2)	10.8	54.20	48.28
Aerobic 2 (pH: 7.2)	11.5	48.90	45.46

pH + Chem: pH after addition of CaO

Table 21: Test of $CaCl_2$ on sample for each stage

	pH + Chem	Tot P initial	Tot P final
Anaerobic 1 (pH: 7.0)	7.2	47.22	33.33
Anaerobic 2 (pH: 7.1)	7.2	54.13	37.61
Aerobic 1 (pH: 7.2)	7.3	54.20	28.27
Aerobic 2 (pH: 7.2)	7.3	48.90	26.06

pH + Chem: pH after addition of $CaCl_2$.

With the CaO, important soluble P removal was made in the anaerobic tank, better than in the aerobic tank which was surprising since it was expected that calcium hydroxide would be formed. It was also not as good as expected given the anticipated coagulant effect of the lime. Moreover, microbial activity would have had been at best inhibited, PAO are reported to need neutral pH (Felipe *et al,* 2001a; Lopez-Vazquez *et al., 2009*).

The addition of calcium chloride also provided precipitation like CaO but without the effect on the pH or influence on the redox. Calcium chloride would also be easier for dilution and distribution. Thus it was concluded that the calcium chloride was a better aid for the milk assisted EBPR. . Calcium chloride has been often used to fortify and increase the calcium in milk for nutrition (Williams *et al.,* 2005).

It was also interesting to observe that the coagulation using calcium produced similar results to the microbial P removal treatment on its own (Figure 28).

5.5.4 Conclusion

Calcium chloride was to be used for the remaining investigations as an adjunct for P removal with milk addition as external carbon source

5.6 Third period membrane bioreactors trials.

5.6.1 Introduction.

There was a delay of one month to liaise with KUBOTA® (one of the major membrane company) to provide new membranes (type 203), and to design and set up a new aeration chamber for the membrane. The biomass from the previous trials were aerated and fed to keep the acclimatised species alive and active with milk. The sludge was well acclimatized to the milk from the previous 3 months experiments and it was intended to reduce possible variations in the nature of the biomass in the sludge. The membrane bioreactor was anticipated as achieving optimal SRT since there would be controlled biomass management simply by removing the excessive sludge.

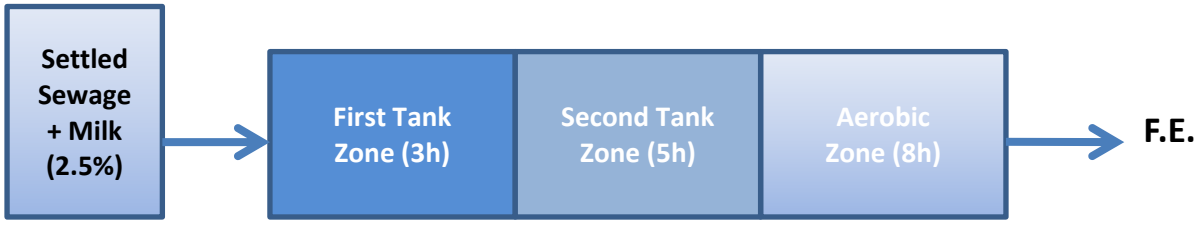
The experiments were sub-divided into different HRT (Anaerobic-Anoxic/Aerobic) as follows:

1. Continuous flow, milk (2.5%), with HRT 3h – 5h – 8h;
2. Continuous flow, milk (2.5%), with HRT 5h – 3h – 8h;
3. Continuous flow, milk (2.5%), with HRT 8h – 4h – 8h;
4. Test on two lines running on parallel without anoxic tank, Continuous flow, milk (2.5%), with HRT 5h – 0h – 8h.

5.6.2 First test series: HRT 3 – 5 – 8.

In this first series of continuous flow experiments, the design HRT was 3 hours for first tank (assumed to have the anaerobic conditions), then 5 hours for the second tank (assumed to have the anoxic conditions), and 8 hours in the aeration tank (i.e., the third tank), see Figure 31.

Figure 31: Continuous flow with (3-5-8) HRT design



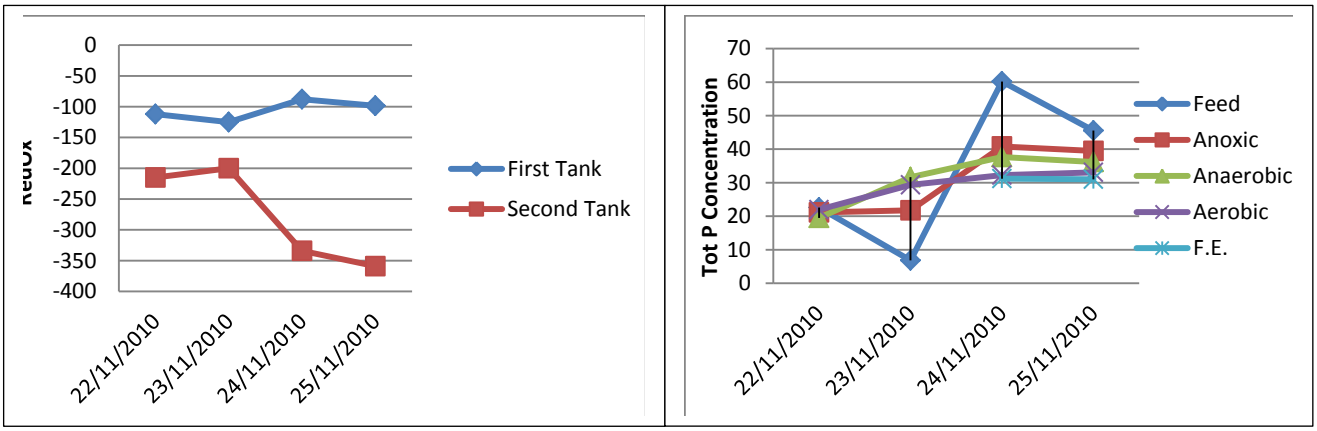
5.6.2.1 Result and discussion.

The process ran for one week before any data was collected. After the 23rd November, as a reminder, the influent had extra phosphorus (in the form of di-sodium phosphate) to the P naturally present in WWTW settled sewage. 2.5% milk was used (25 ml/L feed rate in 10litres/day but also diluted in tap water (50/50). The milk was automatically and gradually, fed directly into the anaerobic tank throughout 24 hours, and so separately from the settled sewage.

Before starting, it was presumed that the anaerobic conditions would have been present within the 3 hours HRT (provided into the first tank, see Figure 31), but the redox potential readings showed otherwise, Figure 32. The RedOx potential was lower in the second tank (designed to be in anoxic conditions).

Figure 32 also shows the phosphorus concentrations of all the supernatants. The tests were carried out only for one week and the membrane from the 24th November, which reduced the turbidity to around 0.3 NTU.

Figure 32: RedOx and P concentration



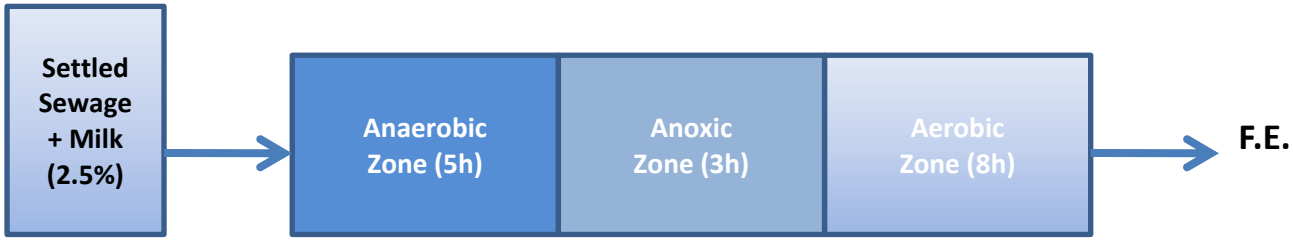
It was difficult to come to any firm conclusion on P release and uptake because the experiment was shortened and stopped, since the phases conditions were never met. The anaerobic RedOx readings were never below -150 mV. The 3 hours HRT was not enough to reach the anaerobic conditions (at least below -300mV) and the RedOx is one of the main factors which determine phosphorus release, the lower the RedOx the more P is released (Rensink *et al.,* 1997).

The results from the aerobic stage were more encouraging and 50 % of the P was retained, both from aerobic supernatant (simple filtration, 11 μm) and F.E. (membrane 0.4 μm) are apparently less than the feed (Figure 32). It was hypothesised that there were still oxidising agents in the anaerobic tank and in very this would negatively affect the POA process (Mulkerrins *et al.,* 2004). The next step was a trial with an extension to the time of anaerobic HRT

5.6.3 Second test series: HRT 5 – 3 – 8

In this second series the anaerobic conditions were extended to 5 hours HRT, Figure 33 shows the trial design. The tank was designed to have an adjustable HRT, and only minor modifications were needed from the previous experiments. A baffle inside the anaerobic tank was removed to give more space. The changes were made on Friday 26th giving the W.E. to adapt the biomass.

Figure 33: Continuous flow with (5-3-8) HRT design



This second period of test experiments was also carried out for a longer period of time but the results were similar to before.

5.6.3.1 Result and discussion.

The Figure 34 describes the RedOx found in the anaerobic and anoxic tanks and it is obvious that anaerobic conditions were achieved also with a lower RedOx than the anoxic zone as a result of increase in HRT. The analysis of soluble P after filtration through filter paper 11 µm pore size for feed, anaerobic, anoxic and aerobic curves are described in Figure 35. The anaerobic phase did not exhibit a release of P in soluble form and in fact the phosphorus was sequestered in the sludge because the level was lower compared to the feed. There was an overall reduction in P to the final effluent but not sufficient to meet the EU target of maximum 1 mg/L allowance.

Anaerobic conditions were achieved and remained steady throughout the experiment.

Figure 34: RedOx potential

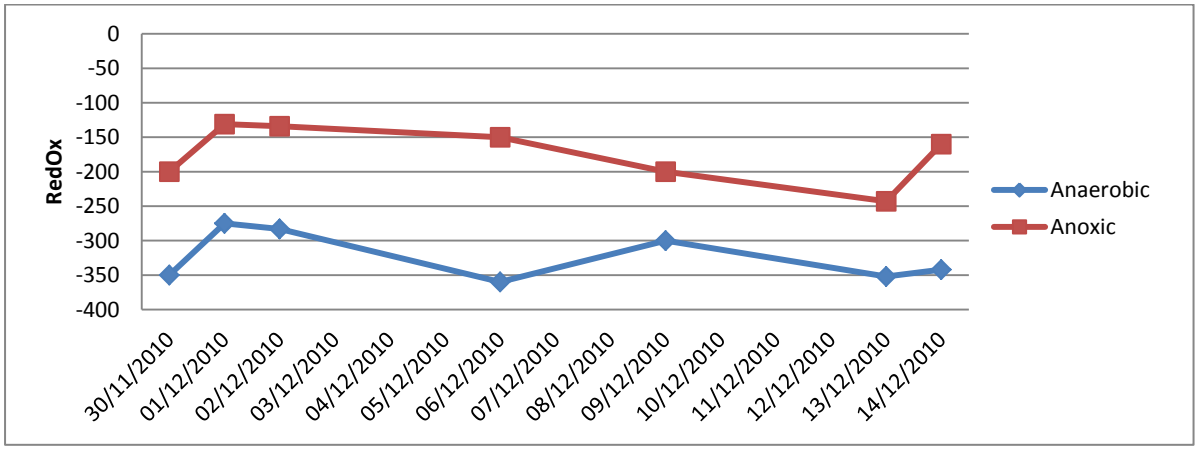
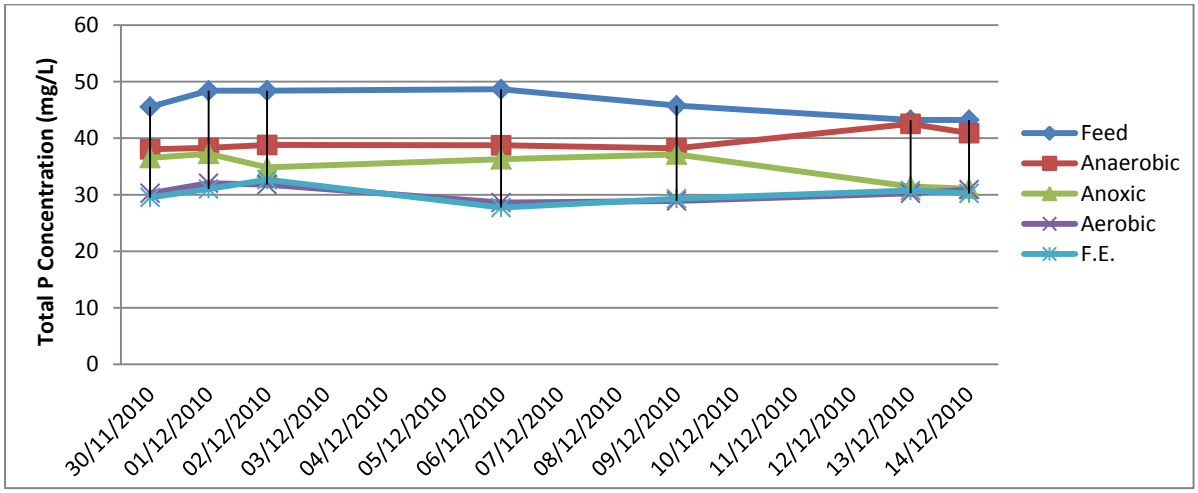


Figure 35: Tot P concentration in supernatant in (5-3-8) HRT design



Two possible explanations were drawn from these experiments. One was that P was removed by direct chelating with milk and sludge particles the other was that the VFAs were not sufficient to promote the PAOs to release the potential P.

Against this however, was the low pH (around pH 4.5) from the anaerobic stage. This environment could suggest excess VFA which could have affected the microorganisms to adjust their internal pH to the environment and thus provoke partial P release. It was also concluded that the P bond to the calcium as described in literature from the milk caseins would have been dissociated at these pH levels (Panouillé *et al.,* 2005) which could prevent ACP formation (van der Houwen *et al.,* 2003; Barat *et al.,* 2011). However the anaerobic curve remained lower than the P in the influent feed.

The pH in the aerobic tank and F.E. was higher, around pH 8.0 and this was where 50% reduction in soluble P concentration occurred. There was little further P reduction after the membrane filtration (Figure 33) and it was concluded the P was soluble or its particles were smaller than 0.4 μm (Kubota® membrane pore size).

5.6.3.2 Conclusion

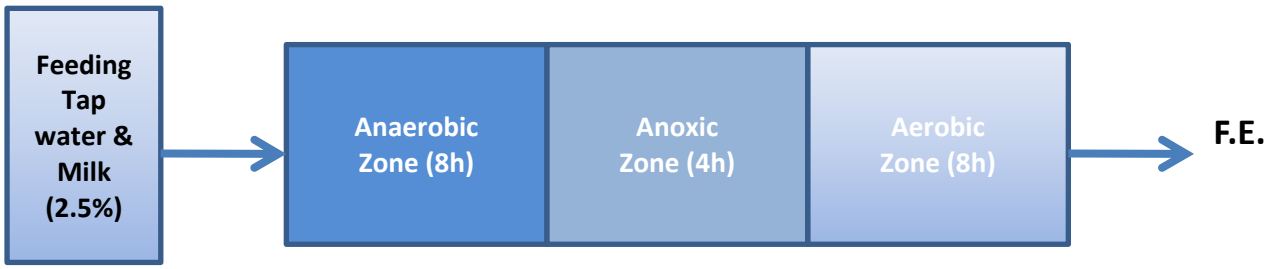
These results were unexpected and not in accordance with the literature on carbohydrate additions making it difficult to assess the role of PAOs. The total soluble phosphorus was removed and the F.E. showed a general decrease. The low pH could be explained by the excess production of VFA from the milk fermentation that was not totally consumed. Thus a further extension to the HRT was carried out to provide additional supporting data.

5.6.4 Third test series: HRT 8 – 4 – 8

The anaerobic HRT was increased from 5 hours to 8 hours and the anoxic HRT from 3 to 4 hours, Figure 36 (the 8-4-8 hydraulic retention time scheme).

Figure 37 shows the soluble P in supernatant after filtration and acid digestion. The experiment has been carried out in two periods either side of Christmas. The system was left working but not regularly fed, rather than stop, clean and restart from scratch. The membrane was stopped in the holiday and was cleaned. A re-acclimatisation period was necessary due to evaporative losses and data collection started again on the 1st February.

Figure 36: Continuous flow with (8-4-8) HRT design



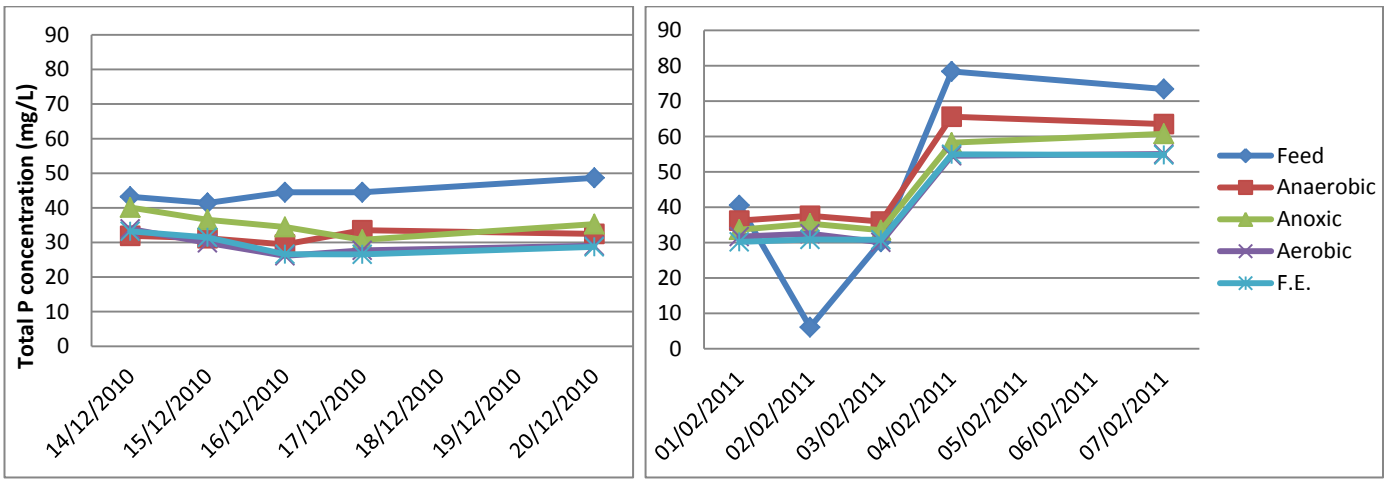
5.6.4.1 Result and discussion.

The results Figure 37 has been divided into these two periods with two different artificial P loads.

- During the first period, P in the influent was almost steady.

- The second period tested an artificial shock load (from 35 – 80 mg/L).

Figure 37: Tot P concentration in supernatant



The system Figure 37 as with previous experiment of this series did not show anaerobic P release or complete uptake of P, the pattern was as the previous experiments. The increase HRT designed to allow more consumption of the VFAs showed no benefit compared to previous experiments.

All experiments have shown a reduction of soluble P in F.E. but not meeting the expectations from the literature or the discharge allowed by the EU. The overall P values are much greater than would be expected in settled sewage but the pattern is also different to previous work. The soluble P content in final effluent was similar to that found in the settled aerobic zone suggesting residual soluble P.

Two explanations for these different results from previous work are put forward as follows:

To obtain the anaerobic conditions, two indicator parameters are possible one is the low RedOx potential around -300 mV. The other is the absence of oxidising agents (e.g., nitrate and oxygen) since nitrate was not regularly measured at this stage because it was assumed that if the RedOx was low then the nitrate would have been lost. The low electronegativity was not easy to obtain, it reached the range of -100mV and -220mV and with hindsight the reduced heterotroph bacterial activity may have been the problem since the nitrate could not be involved in absence of recycle loop between anoxic or aerobic tank.

The soluble P in the anaerobic tank did not increase when it was assumed that the colloidal calcium phosphates and some other precipitates should have been partly or completely dissolved and re-released according to Barat *et al.,* (2011). The addition of milk and acidic conditions, the pH was around 4.5, should have released even more soluble P. Le Graet & Brule (1993) reported that the total mineral phosphorus from milk became completely soluble at pH 5.2. In work carried out by Smolders *et al.,* (1994a) on acetate uptake by PAOs in anaerobic conditions they stated that even at low pH, PAOs still released low P and this carried on until pH 4.5 and thus close to our pH. Our results are contrary to these observations as the soluble P was reduced compared to the feed (Figure 37).

One possible explanation, the original hypothesis of the thesis was that the milk chelating agents had a stronger binding potential than pH or RedOx, keeping the P in the sludge. In milk, inhibitors were identified in the literature review that prevented the calcium phosphate aggregation and then precipitation. Schmidt and Both (1987) identified the β-casein fragment 1-105, which concurred with later observations made by Van Kemenade (1989a) from the caseins αs, β and κ. However it was supposed that these proteins would be hydrolysed after fermentation and therefore not escape the anaerobic hydrolysis process. The results here however suggested this was a possibility again due to the low bacterial activity.

5.6.4.2 Conclusion

In the anaerobic zone the P release was not achieved and it was concluded either as a result of insufficient low RedOx or from the hypothesised existence of binding agents in the milk. In this configuration the benefit of anoxic activity was not obvious therefore it was envisaged in the next experiments to exclude the anoxic tank from the system to better define its role in soluble P removal. The 8 hours anaerobic HRT did reduce the anaerobic electronegativity but the effect on VFAs was uncertain. It was therefore decided to return to the previous HRT (experiment 5.6.3.0) which achieved better P release but without the anoxic tank to make interpretation of the results easier.

5.6.5 Fourth test series: HRT 5 – 0 – 8

Usually the anoxic zone is used to remove nitrate that would otherwise raise anaerobic RedOx potential, but in these experiments there was no aerobic effluent recirculation and thus nitrate. It was also assumed that all organic nitrogen in the feed would have been removed if the RedOx potential in the anaerobic zone achieved the desired level.

The laboratory design and previous experimental results made it clear that the anoxic zone had become an anaerobic extension during the attempts to get P release and that PAO behaviour was not changing. It was possible that the organic nitrogen in the milk was releasing too much oxidizing agents. This experiment bypassed the anoxic treatment zone of the system to use the simpler anaerobic (5h) and aerobic (8h) scheme.

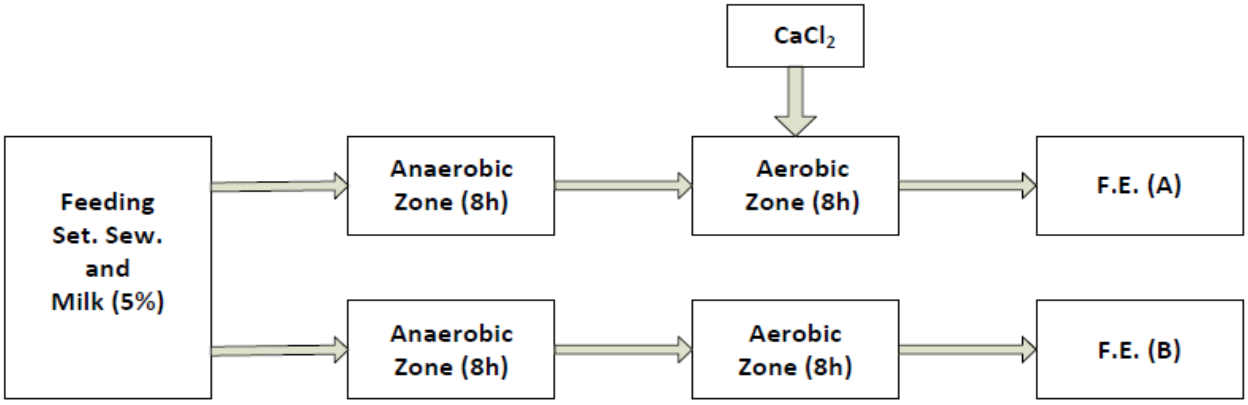
For the first time in the study two treatment trains had been built and could run in duplicate mode, one as the Control Line (B) and the other as the Test Line (A). Therefore it was also possible to investigate calcium chloride additions to reduce the solubility of the super saturated P. The $CaCl_2$ was added to Test Line (A) (Figure 38) with the same feed for both lines.

The decision to choose calcium as coagulant was decided by the results of the literature review and the preliminary tests in Section 5.5. Moreover, biologically induced calcium phosphate leads to complex scale and struvite formation with further precipitation. These are frequently reported in the literature to be the most important precipitation processes in wastewater containing high concentrations of calcium and magnesium (Barat *et al.,* 2011). The experiment ran from the 7th February, and 300 ml (250 mM) $CaCl_2$ was manually added into the aerobic tank. Data collection started on day 2, the 08/02/2011, for the 09/02/2011.

The first 2 days (7-8 February) were used to measure the reproducibility of performance between the two treatment trains Line (A) and Line (B) (control) before the addition of calcium chloride. An excess of calcium was deliberate to enable easier observation of the changes. Research by De Kort *et al.,* (2009) on calcium binding capacity of organic and inorganic phosphates in activated sludge was used as a reference.

They precipitated 100 mmol.L^{-1} phosphates with 50 mmol L^{-1} $CaCl_2$. Their research was made with synthetic sewage containing special MLSS made of a complex variety of competitive organic and inorganic particles chelating ions.

Figure 38: Continuous flow with (8-0-8) HRT design



5.6.5.1 Result and discussion.

The pH was in the range of 5.2 – 6.9, and the RedOx, for both anaerobic tanks, was around -300 mV, it was increased slowly during the experiment.

The soluble P concentration of Line B (without $CaCl_2$) is presented in Figure 39 and Line A in Figure 40.

Figure 39: Control line B (without $CaCl_2$)

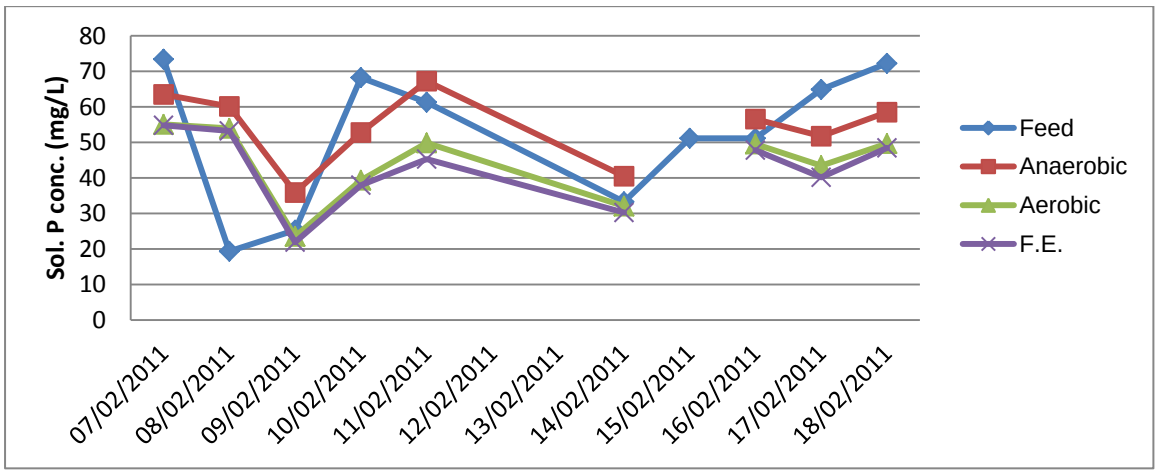
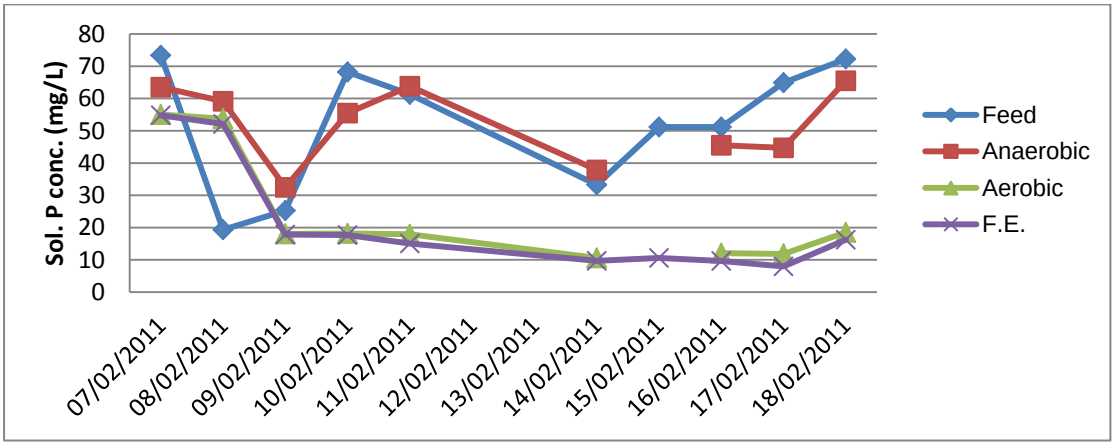


Figure 40: Testing line A (with $CaCl_2$)



The feed concentration curves are identical because the influent was from the same tank. The soluble P content from the anaerobic tanks was almost identical and on average their curves followed a similar pattern to the feed as in previous experiments. The main differences between Lines (A) and (B) came from the aerobic and final effluent curves namely as a result of the $CaCl_2$ addition. This observation was encouraging because the addition of calcium chloride occurred in the aerobic tank, suggesting that the soluble P bound to the calcium provided. As was noticed from previous tests, there was no important demarcation between the aerobic curve and F.E. curve in either lines. The final effluent has been filtered by the 0.4μm membrane pore size and the aerobic supernatant, filtered by an 11μm pore size filter. It was the magnitude of difference that was important in line (B) F.E. which was always between 30 mg/L and 40 mg/L whereas, F.E. line (A) was always below 20 mg/L after calcium addition. However, both lines failed to achieve the 1mg/L final effluent discharge.

5.6.5.2 Conclusion

Regardless of the arguable fact that it could be stated that the concentration of calcium chloride was high (200 ml/day, 250 mM concentration), the results showed that it is an effective method of precipitation of soluble P. The fact that the aerobic and the F.E. curves were almost identical seems to point out that the remaining phosphorus is mostly soluble (smaller than 0.4 μm). Larger sizes (e.g., 1μm) are known to be successfully trapped in activated sludge as large colloids (Tchobanoglous *et al.,* 2003). The membrane could be beneficial for removing the 1 – 0.5 μm colloids but this was not properly investigated by an alternative solids/liquids separation process. The still high soluble P concentration despite the injection of Ca^{2+} suggested the hypothesis that either P was in excess of the available calcium or that new ACP species were formed but not aggregate and thus remained soluble.

5.7 Final discussion

The soluble P data from anaerobic stage showed no release of P by the PAOs at any stage and thus following the standard theory in the literature this would have affected the P re-uptake during the aerobic stage. The theory is that this P release generates PHA, PHB and PHV stored as accumulated substrates during the anaerobic stage from the take up VFAs (Mino *et al.,* 1994; Mino *et al.,* 1998). A possible inhibiting influence was pH. Smolders *et al.* (1994a) found that when the pH decreased, the amount of P release by PAOs also decreased as a result of the detrimental effect on EBPR and PAOs growth in acidic environment. This is related to the energy necessary to assimilate the substrate across the cell's membrane which is higher in alkaline environment (high pH). Therefore, according to the proton motive force (PMF) hypothesis, more energy is required to take up VFA, and in alkaline conditions more intra poly-P must be broken down, and then released in the surrounding, to generate it. Smolders *et al.* (1994), Filipe *et al.* (2001), Jenkins and Shuler (2002) all stated that a higher pH in the anaerobic zone was beneficial for PAOs and reduced the competitive advantage of GAOs.

Results from all the experiments showed a low pH in the anaerobic tank and this could be explained by the high concentration of organic acids after milk fermentation. The hydrolysis of proteins, carbohydrate, and fat that compose milk, provided a large amount of lactose, amino acids and volatile fatty acids. It is these acids such as lactic acid bacteria in milk that are assumed to produce the low pH. Furthermore, if the third stage in anaerobic fermentation known as acetogenesis also occurred during the redox and HRT, it could have reduced larger acids into acetic acids (Demirel *et al.,* 2005; Hassan *et al.,* 2012).

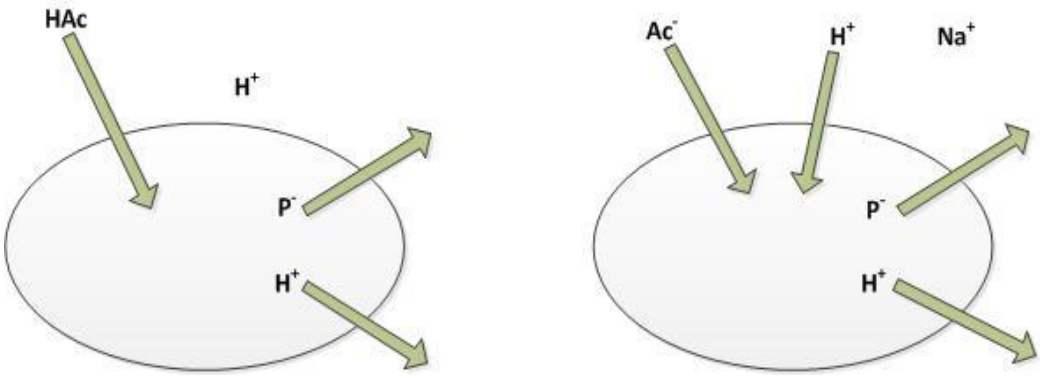
However, other experiments from several researchers studying the pH effects on EBPR efficiency gave almost opposite results for the same range of pH (Randall *et al.,* 2012). Fleit (1995) also did not agree with the Smolders et al. (1994a) hypothesis mainly because, according to the microbiological literature, acetic acid (ACE) does not require energy to diffuse across the cell membrane and thus it is not necessary to break down the polyphosphate to create energy. They used acetic acid for their experiments to confirm this hypothesis. Fleit (1995) explained that the P release was made to balance the internal pH by releasing a proton (H^+) along with phosphorus making the acidic environment less effective since the microorganism must reuptake the proton (H^+).

Another similar theory for the phosphorus release mechanism was proposed by Filipe and Daigger, (1998) who proposed that during the anaerobic conditions the PAOs absorb, for example acetic acid, to balance their internal pH with the external environment, and thus generate a higher internal proton motive force (PMF). Releasing P with an H^+ ion maintains the equilibrium (Filipe and Daigger, 1998).

Recent research made by Randall *et al.* (2012) restated that the pH variation during the anaerobic phase had an upsetting effect on the EBPR. They were able to differentiate between if the adjustment was made with an inorganic or organic acid (or base). Organic acids produced a better net phosphorus removal but when made with inorganic acids (or base) removal declined. The second observation they reported was that acetic acid was superior compared to its base form, sodium acetate, as supplemental VFA to improve EBPR performance (Figure 41).

The hypothesis is that the inorganic acids provoke a homeostasis reaction which forces the cell to excrete P^- along with the proton H^+ (called symport) in order to maintain its internal pH, whereas addition of organic acids release the ions P^- and H^+ to keep the proton motive force (PMF) equilibrium of the cells, and this latter process is better accepted by the PAOs.

Figure 41: Comparison of acetic acid and sodium acetate (NaOAc) for P release (Randall et al., 2012)



After P release, the internal pH remains neutral and for the maintenance of PMF, the P release for HAc is greater than Ac^-.

Randall's work on pH showed that more than the pH level itself, the conditions that change the pH environment are a more important influence on EBPR efficiency. Therefore in this thesis we have two different milk concentrations, high and low, to create a change in the pH environment. If our EBPR efficiency remains as good at lower as higher milk concentration then this would support Randall et al. (2012) conclusions that the type of acid was important.

Randall *et al.* (2012) also suggested that if rich mixed VFA acids entered in the anoxic zone providing adequate substrates to both the PAOs and DPAOs, the anoxic tank could promote P release and synthesise PHA to replenish the glycogen pool within cells. The anaerobic and anoxic tanks acted similarly despite the difference in redox. Therefore in the next experiments it was decided to have a recycling loop between the anoxic and the aerobic tank.

Other experiments, such as Oehmen *et al.* (2005a, 2006a, b), made in standard conditions (such as temperature close to 20°C and pH 7.0 for example), showed that the take up of VFA can be similar and at the same kinetic rate for both PAOs and GAOs. If the negative effect of low pH (lower than pH 7.5), or experiment in situations where the carbon source is specifically for example made of propionate or acetate only which could give specific advantage to one microbial species over another, or if acidification/alkalinity was controlled inorganically rather than organically, then the results found in literature could also have been biased according to Randall *et al.* (2012).

Low anaerobic pH is reported to be detrimental for EBPR in general texts (Tchobanoglous *et al.,* 2004) however, in our experiments, the low pH in the anaerobic zone did not directly affect the re-uptake of P despite that the PAOs were not recycled in the anaerobic tank. A decrease in soluble P still occurred during the aerobic phase, but the contrast was even more obvious when the lines were operated similarly in the same condition with one line only receiving the additional $CaCl_2$ (see Figure 39 and Figure 40).

Providing more $CaCl_2$ (done in the aerobic tank) was supposed to provide excess Ca^{2+} to chelate free PO_4-P and soluble P. According to De Kort *et al.* (2009) most of the calcium phosphate formed in the experiments should have the favourable conditions to precipitate. It was noticed that there was almost no distinction in phosphorus concentration between the filtered samples collected in the aerobic tank (11 µm pore size) and final effluent tank (0.4 µm pore size), suggesting that the P was mostly soluble or the small colloidal calcium phosphates (< 0.4µm) were too small to precipitate or to be removed by the membrane.

Thus overall the results suggested that the role of PAOs was reduced. This could be due to the increased activity from the milk resulting in all the P being solubilised more quickly or from the linked low pH. There was also evidence of special calcium and phosphate chemistry in milk would augment the coagulating properties of calcium additions. .

5.8 Conclusions.

The original hypothesis was that milk addition would improve PAO growth by both providing a source of readily biodegradable carbon and additional sustainable complexing agents for the phosphate.

1. PAOs were grown successfully and were increased in the laboratory experiments by using standard skimmed milk.
2. The chelating agents that were hypothesised to be present in milk proved to be difficult to identify separately from calcium in milk. It was concluded that Ca and PO_4 might have been more significant for nucleation.
3. Calcium chloride addition enhanced otherwise limited P removal.
4. The membrane pore size of 0.4μm, excluded typical larger wastewater particles (e.g.: microorganisms, colloids, solids etc…), and did not show clogging. The analysis of F.E. samples showed almost completely removal of particles with very low NTU (Annex VI) and also very low TOC (Annex I and II).
5. The MLSS achieved in these first continuous flow experiments were 30% of those anticipated in an operating MBR suggesting steady state was not achieved. The experimentally exaggerated concentrations of P however overcame any doubts in precision of the analytical results and gave the necessary confidence in the experimental variables; length of anaerobic period, shock and steady P loads and nucleation.

5.8.1 Recommendations for next tests

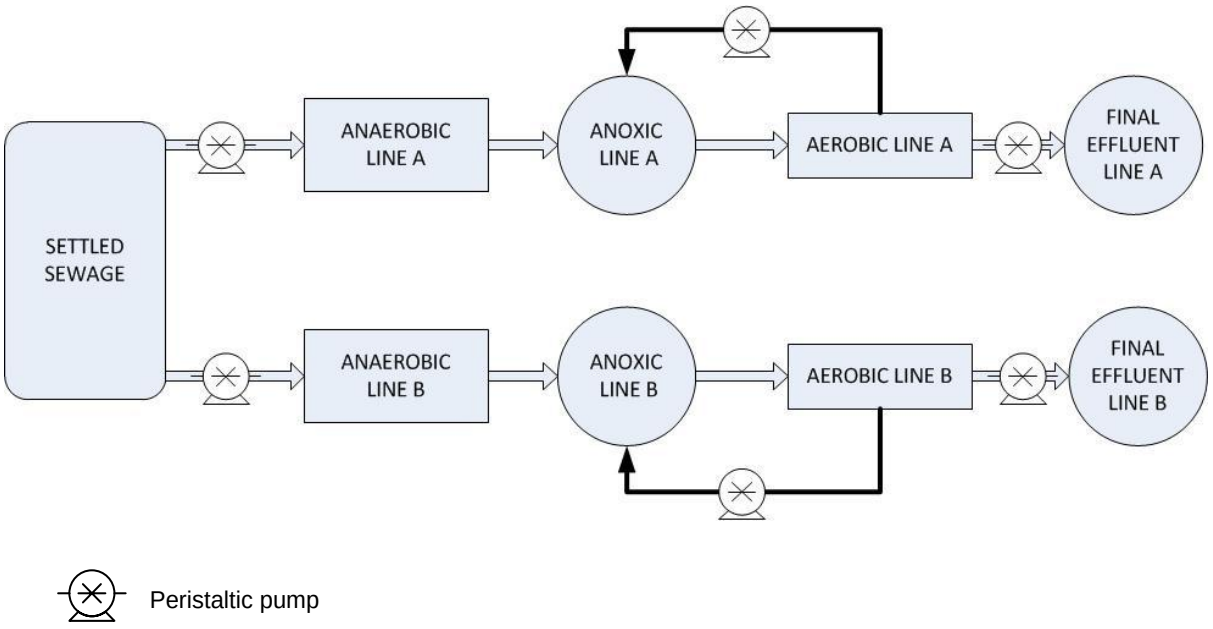
1. Design improvements for the next stage included a recycle loop between the anoxic tank and the aerobic tank as exposing the PAOs to different environments should improve the biological removal of P. This was not used in trial Phase and also adding an extra recycle loop would make the system more comparable to the traditional University of Cape Town process model (UCT).
2. An extra physico-chemical dose as a final treatment stage consisting of a stirred tank for the final effluent initially seeded with $CaPO_4$ to avoid competition for the calcium in the activated sludge and improved the removal of the soluble P.

6.0 Laboratory continuous flow rig tests

6.1 Introduction

Compared to the last experiments, this design has included again the anoxic tank but with a recycle loop from the aerobic tank. This design remained unchanged until the end of the research, as described by the schematic shown in Figure 42.

Figure 42: Double line laboratory continuous flow system



The two experimental lines A and B (Figure 42) included a control. The recycle loops have been installed between the anoxic and aerobic tank to favour the growth of PAOs by helping the heterotrophic microorganisms to ferment the COD anaerobically and thus to enhance the EBPR. Four main reasons help the decision for that single loop only:

- Firstly, this recycling method avoids the introduction of nitrate in the anaerobic tank that could help the development of anaerobic denitrifyers. Since they consume substrates and do not ferment the potentially soluble substrates into readily available substrates, they would therefore reduce the easily assimilable substrates to PAOs (literature review 2.7.1.2) (Henze *et al.,* 1995; de Lucas 2007, a, b).

- Secondly, by using the membrane, it was possible to retain the sludge for a longer time, helping the growth of slow-growing organisms such as the PAOs (Smolders *et al.*, 1994a; Silva *et al.,* 2011).

- Thirdly, by exposing the PAOs to NO_3^-N in the anoxic tank, the process will stimulate the PAOs metabolism as they are capable to denitrify by adaption and have the same metabolism as DPAOs. It was not the intention to distinguish between DPAOs and PAOs in this study although it is reported in the literature that they are the same micro-organisms (Zheng *et al*., 2003; Carvalho *et al.,* 2007). Others state that different groups of PAOs are also able to use NO_3^-N as an electron acceptor to uptake PO_4-P (Freitas *et al.,* 2005, Flowers *et al.,* 2009, Garcia-Usach *et al.,* 2010).
  This hypothesis was to be confirmed by this experiment as it is necessary to expose DPAOs to NO_3-N to stimulate the denitrifying biochemical pathway and induce the necessary nitrate reductase (enzyme to reduce nitrate) and the oxidation enzyme to uptake phosphorus was always present. Generally full scale plants monitoring nitrogen reduction also had a good P uptake in the anoxic zone (Kuba *et al.,* 1996a).

- Fourthly, the very low pH that was found earlier during the trial experiences, although controversial in the literature, would not have favoured the PAOs and P release in the anaerobic tank. The low pH would have reduced the possibility to release PHA for P uptake within anoxic and aerobic zones. Moreover, considering that the ordinary heterotrophic organisms (OHO) can represent up to 95% of the total bacteria population in the activated sludge, and that they are better equipped to thrive in many environments thanks to their large biodiversity, but also because OHO are bacteria capable to ferment organic substrates and also reduce rbCOD into VFAs (Lucas *et al.*, 2007b). Therefore, it was hypothesised that the more useful slow growth PAOs would have been more efficient if receiving an effluent rich in VFAs from the anaerobic zone and at neutral pH rather than competing in a hostile environment.

6.2 Experiment Phase I

6.2.1 Introduction

Phase I was the first part of the investigation and run for 6 weeks to find the optimum concentration of milk to be injected that would improve the biological removal of soluble P. Another important aspect was to avoid creating excessive growth that would increase the sludge removal occurrence, the oxygen demand and the membrane cleaning necessity. The phase was separated in two periods of 3 weeks. It was envisaged from the earlier experiments to evaluate 5 and 10% of milk concentration but after few days of experiments, at 10% milk concentration it was apparent that it would have been harder to manage. Difficulties such as foam formation in the aerobic tank, a rapid increase of MLSS in every tank and turbidity in the final effluent led to decision not to analyse and abandon the idea of trying these experiments.

A glucose mix was injected in a parallel line to be used as another carbon source. The COD concentration mirrored the milk COD concentration (see Section 6.2.3.1 below in feed characteristics for the details). This parallel line was used as control to assess performance efficiency.

6.2.2 Material and methods

Both lines received 200 ml of 250mM $CaCl_2$ concentration to be injected in two different places, one in the anaerobic tank and the other one in the aerobic tank both at 100ml/24h flow rate.

6.2.2.1 Lines experiments characteristics

- Line A feeding: Settled Domestic Sewage with milk enrichment (Long life semi-skimmed)

- Line B feeding: Settled Domestic Sewage with Glucose, Dextrin and Starch.

6.2.2.2 Feed characteristics

The settled domestic sewage was collected from the local wastewater treatment work in Loughborough every Friday in order to keep the same conditions during the week. The differences between the lines (A and B) were in the type of feed enrichment, but in order to keep the same COD strength at the starting point (before the anaerobic zone) the COD of glucose mix was measured to have the same value as the milk concentration. The semi-skimmed milk was used during the experiments to reduce the problems with fat on the membrane, but also to aid the filtration of samples collected in different zones.

Long life milk was also used to reduce the tendency to turn to curdle with age. Characteristics of the milk used are in Table 36, Annex V.

6.2.3 Results and discussion - Phase I: First Period

This first recorded period was done for 3 weeks (from 23/05/2011 to 10/06/2011). **Line A** used 1% milk concentration while **line B** used the glucose mix.

6.2.3.1 Milk concentration equivalence

Glucose mix to be equivalent to the 1% milk concentration: 3g Glucose – 1.5g Dextrin - 1g Soluble Starch (diluted in 100ml tap water)

6.2.3.2 Collection of settled sewage in Loughborough WWTW

- 200 litres were collected at the same time to keep the characteristics for many days (Friday 20/05/2011 used same day until the 02/06/2011)

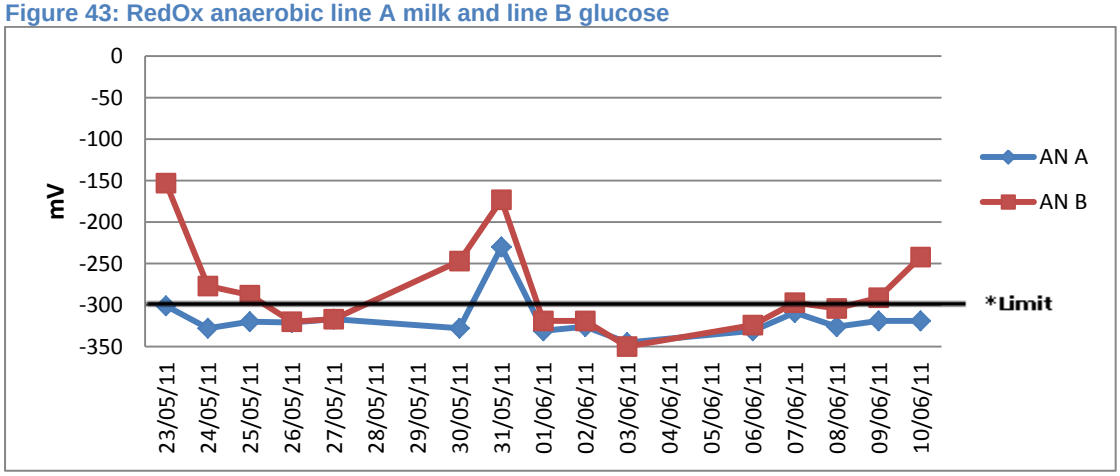
- 125 litres were collected at the same time to keep the characteristics (TOC strength, dissolved organic carbon, soluble P etc. …) for many days (Friday 03/06/2011 used on Monday 10/06/2011)

- Line (A) milk and line (B) glucose mix were fed, and supplemented with P added separately and directly into the **anaerobic** tanks.

6.2.3.3 RedOx from line A and line B.

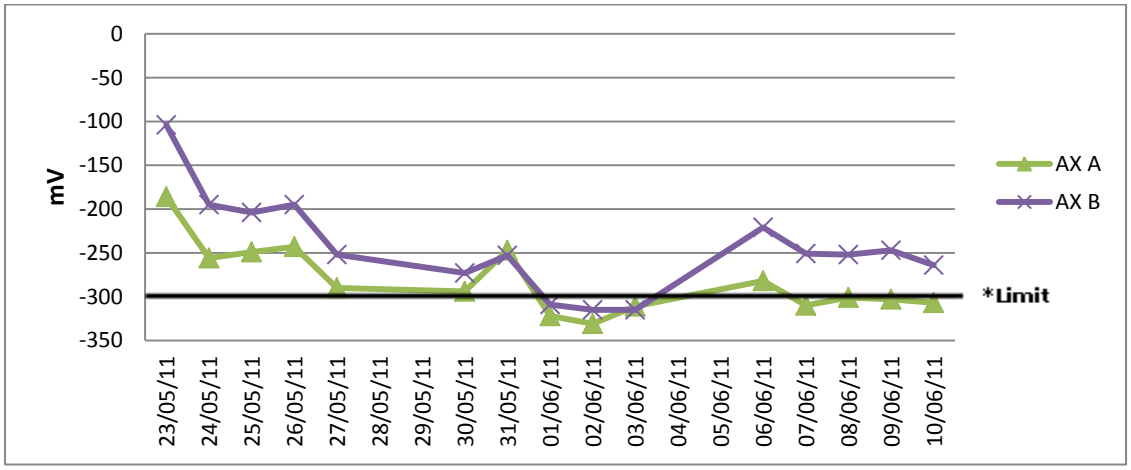
Figure 43 presents the RedOx from anaerobic line (A) milk and line (B) glucose. Figure 44 shows the anoxic RedOx from the same lines.

Figure 43: RedOx anaerobic line A milk and line B glucose



* Limit is the border between anoxic (0 mV to - 300 mV) and anaerobic (-301mV to - 500 mV) condition.

Figure 44: RedOx anoxic zone line A and B



* Limit is the border between anoxic (0 mV to - 300 mV) and anaerobic (-301mV to - 500 mV) condition.

Line (A) milk easily met the anaerobic conditions (Figure 43) prescribed, except once (day 31) because of a power cut. Since different pumps were used, the extra carbon supplement pumps did not restart (by default), whereas influent pumps resumed the feeding. Therefore, the anaerobic MLSS was diluted and reduced the TOC strength. The anaerobic conditions in the line (B) glucose were met only 50% of the time. For the anoxic reactors, line (B) glucose was always defined as anoxic zone, and line (A) milk was either similar or slightly lower. It was noticeable that the RedOx potential in the anaerobic zone was not much lower than the anoxic zone when figures 43 and 44 are compared.

6.2.3.4 pH from line A and line B

Figure 45: pH anoxic and anaerobic line A

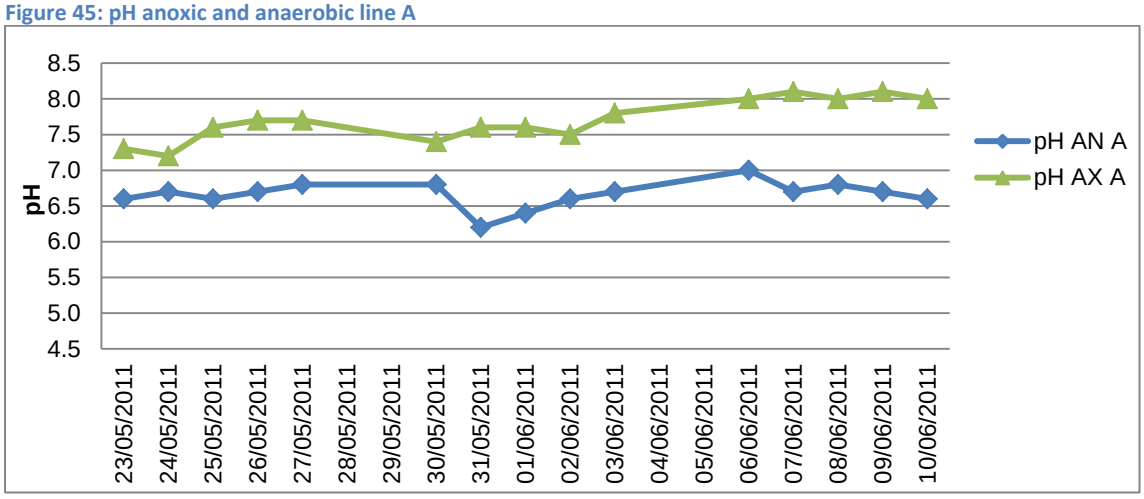
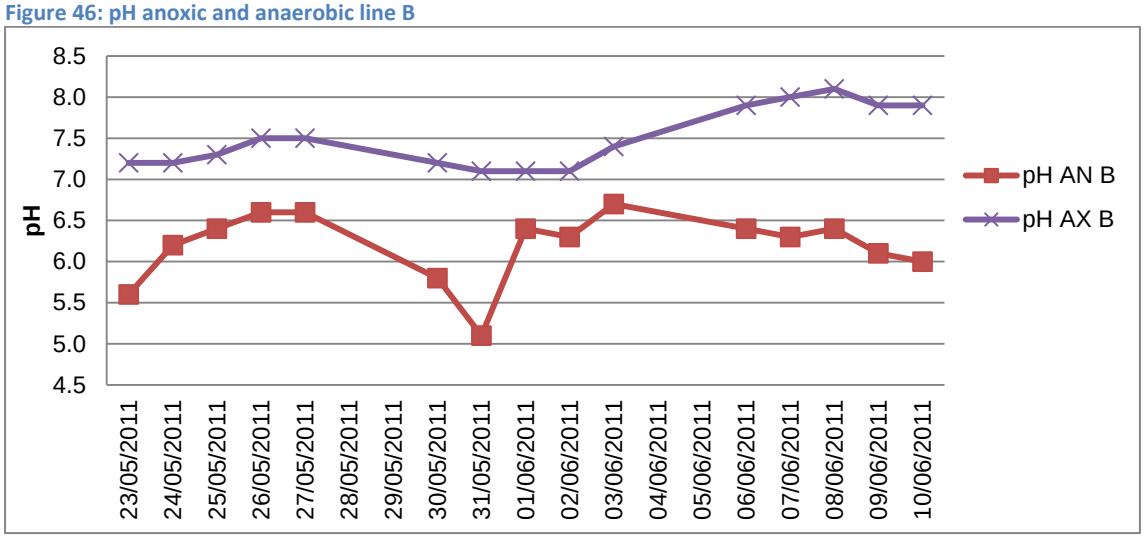


Figure 45 presents the pH for the anaerobic and anoxic zone line (A) milk. The pH was quite steady for both zones (between 6.5 and 7.0) and very close to the milk pH (6.8). The neutral pH in the anaerobic zone should procure good conditions for the microbial growth.

The pH for the anoxic line (A) milk increased slowly to stabilise around 8.0, (Figure 45) a pH level between 7.0 and 8.0 was reported in literature to be optimum for most denitrifying bacteria to strip the nitrogen (Wang *et al.*, 1995). During a power cut, the anaerobic pH decreased towards pH 6.0 (30/05/11) in the milk reactor (Figure 45) and to 5.0 in the control reactor (Figure 46). A 2 day recovery period was required for the anaerobic reactors to reach the previous pH condition whereas the anoxic reactors were not affected .

Figure 46: pH anoxic and anaerobic line B



The pH in the anaerobic zone line (B) glucose (Figure 46) was lower than the test milk reactor and close or below pH 6.5. The glucose mix as might be expected was creating more acidity in the anaerobic tank than the milk as exogenous carbon. The effect of the power cut was magnified in the glucose reactor and have been attributed to the small molecule weight of glucose and simple conversion to VFA.. These low pH levels concurred with other experiments where low pH levels have also been found for example (Kargi & Uygur, 2004; Gebremariam *et al.,* 2012). The power cut has also reduced the pH level as in the milk carbon dose, but less significantly (Figure 45) the anoxic pH in both reactor trains was hardly affected.

The pH from anoxic tank line B was stable for the first 2 weeks and neutral, between 7.0 and 7.5 but increased the last 3rd week, from the 2nd June again. The increasing MLSS concentration in the anoxic tank may explain the pH stability even when there was acidity discharged from the anaerobic zone but also stripping the nitrate increase the pH.

Both results reported in Figure 45 and Figure 46 showed how the power cut affected the results magnifying the extra carbon coming from the glucose rather than milk. Figure 46 demonstrating the risks of VFA from simple sugars and supporting the results from literature.

6.2.3.5 Nitrate concentration.

Before the 2nd June (23/05 – 01/06), a lot of tests and trials were made through flow adjustments, and therefore making several changes in HRT during short period of time. The nitrate in line (A) milk started high at 120 mg/L (25/05) during this test (Figure 47) and remained high, but the interesting point was on the 30th May when the power cut deprived the system of oxygen. The nitrate concentration in line (A) milk improved and became comparable with the glucose line (B), however with more variability.

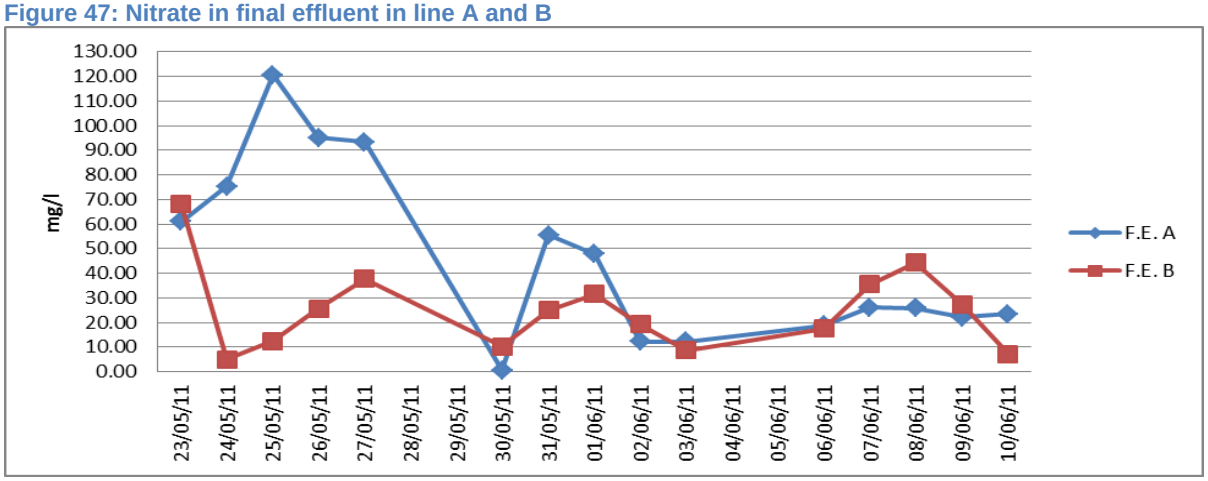
Since the nitrate concentration has reduced in both lines and dropped below 1 mg/L, the results suggested that increasing the HRT in the anoxic zone would improve denitrification, and by implication, the P removal. From the 2nd June the HRT in anoxic tank increased from 6 to 8 hours and the aerobic tank HRT reduced from 8 to 6 hours. Figure 47 demonstrated that denitrification was improved after this point.

The nature of the carbon source could be very important in explaining the rate of NO_3 where the glucose mix was yet readily absorbable for denitrifying bacteria compared to the milk allowing a lower level of NO_3-N. However, after the power cut there were changes in behaviour and two explanations were still possible:

1. First was the extension in anoxic HRT which provided more contact time for the bacteria to reduce the nitrate and become adapted to their environment. OHOs and DPAOs had no other choices than use the nitrate to take up the VFAs increasing the bacteria pool in the anoxic tank;
2. Second was based on an anaerobic explanation where milk fermentation improved with ages, as it was explained in the literature review, milk could provide enough VFAs but needed time to be completely hydrolysed into small molecular weight organics. After 30 hours VFAs were still released from the milk whereas glucose fermentation was very rapid, in less than 2 hours (Lucas *et al.,* 2007b).

The combination of both explanations might be correct as the nitrate reduction improvement enhanced with time despite the greater total amount of N in the milk line because of the organic N in milk. Therefore, the fresh milk supply combined with the fermentation, resulted an effluent richer in VFAs from the anaerobic tank that improved the denitrification. Even with a high level of N from using milk, increasing the concentration as extra carbon was still considered beneficial without causing problems. The power cut did probably stimulate the bacteria since there was no choice but to use the nitrate available. Both lines had optimum pH values (between 7.0 and 8.0), where denitrification is reported to work better when in alkaline conditions (Wang *et al.,* 1995; Glass & Silverstein, 1998).

Figure 47: Nitrate in final effluent in line A and B



6.2.3.6. pH in aerobic tank and final effluent.

Figures 48 and 49 show the pH measured in the aerobic and F.E. of the two reactors.

Figure 48: pH aerobic and F.E. line A

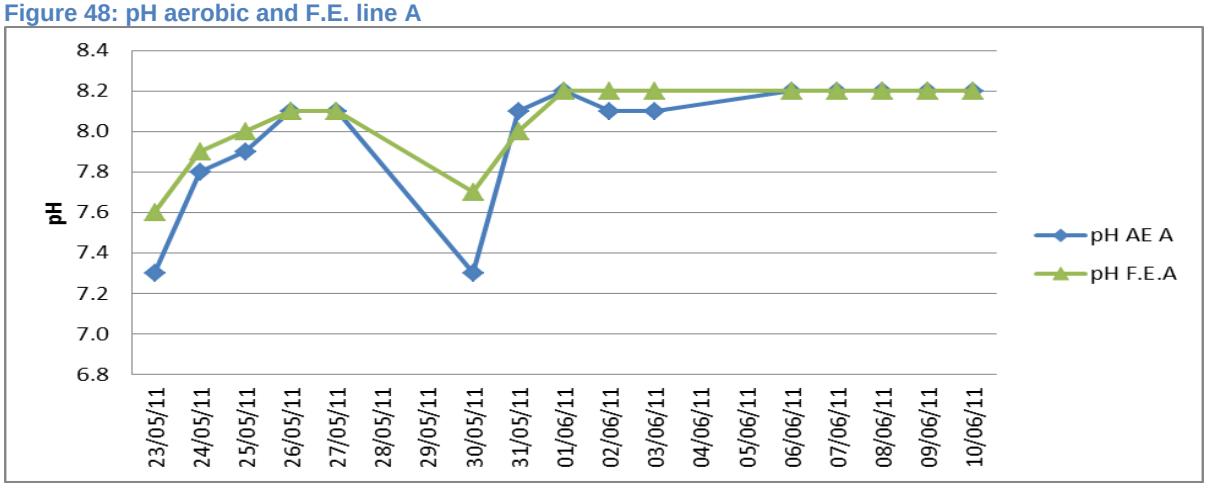
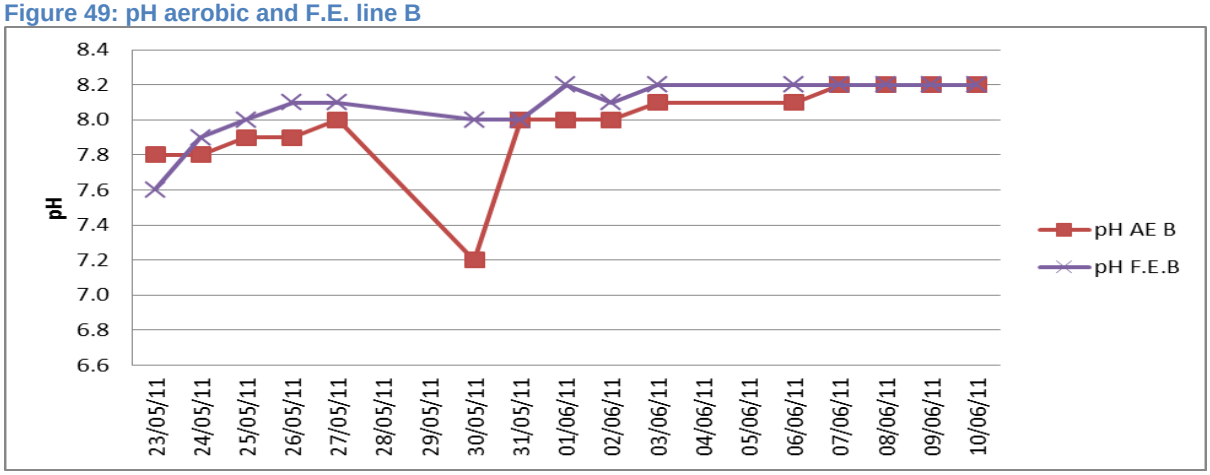


Figure 49: pH aerobic and F.E. line B



Apart from of the power cuts date (30/05/2011), the aerobic pH line B glucose rose linearly between 7.8 and 8.2 during the experiment. The pH with the aerobic milk line A started at lower level but was stable at 8.2, and the final effluent follows the aerobic curve pattern as it was supposed to do without further reactions.

6.2.3.7 Phosphorus

6.2.3.7.1 Introduction

The concentration and evolution of phosphorus was measured at each zone, every day (except the W.E.) to assess the release and uptake. The results for the final effluent are discussed later in this section.

The concentration of phosphorus in line (A) milk and line (B) glucose for the feed, anaerobic and anoxic zones are summarised in Figures 50 and Figure 51. The curve labelled “Feed” is the wastewater settled sewage filtered supernatant. The same settled sewage has been used in both lines and thus soluble P curves are identical.

The 28th to 30th May (weekend to Monday) power failure gave a break in pattern of the curves, and no aerobic sample was collected from the aerobic A & B reactors on the day 30/05/2011 because there was no air provided, the aerobic zone became anaerobic.

A manual vacuum pump was used to separate the liquid from solids with a filter paper 11 μm until the 27th May for anoxic samples, and until the 9th June for anaerobic samples, because it was difficult to have liquid by simple gravity, however the filtrates looked murkier. The acid digestion was used (see the method in section 4.5.3.1) which means that it is certain that the level of measured P has been overestimated, because solids passed through the filter and contaminated the soluble P in the supernatant. This manipulation was made for both lines (A) and (B).

6.2.3.7.2 Phosphorus content before final effluent

Figure 50: Phosphorus concentration in line A milk

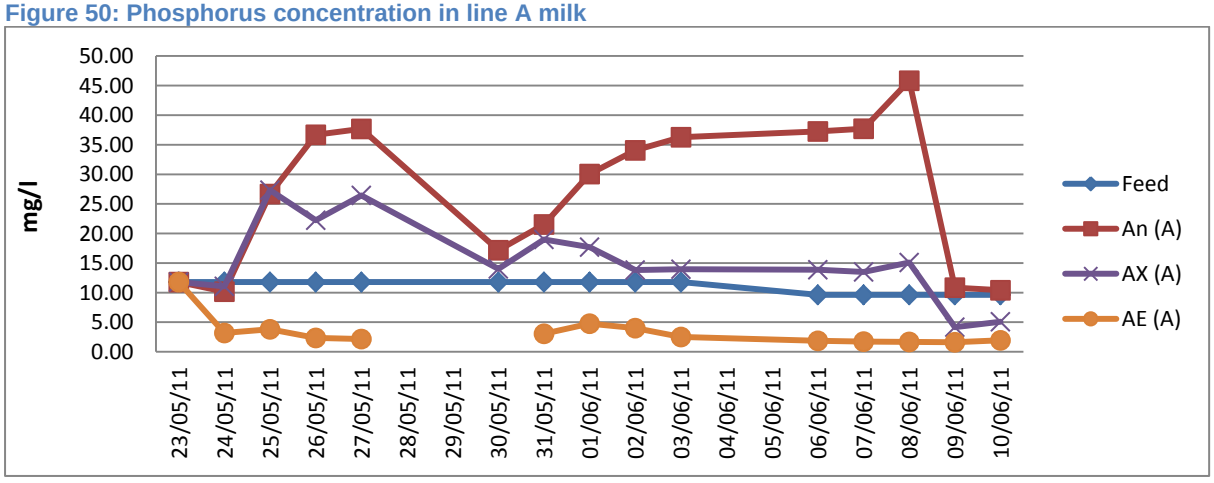
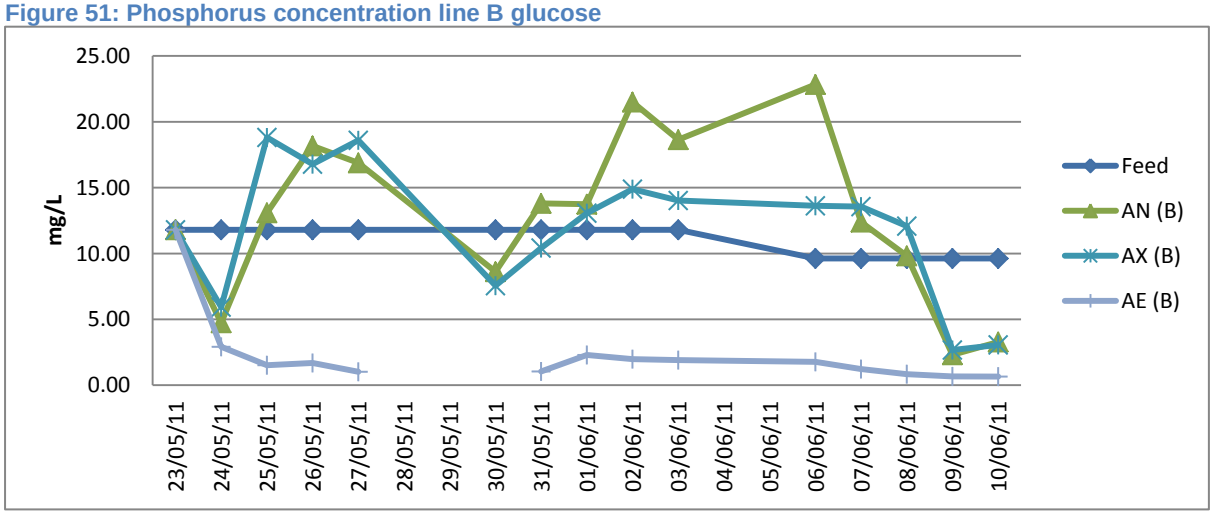


Figure 51: Phosphorus concentration line B glucose



The phosphorus concentration in anaerobic supernatant line (A) milk is higher than the control most certainly due to the P in milk compared to line (B) where glucose does not bring additional P to the sewage.

The sudden change in P on the 9th June was unfortunately, at a time when there were enforced changes in technique. One was the change in analytical machine (see 4.5.1) but also the pore size of the filter technique was changed as noted. The results from the aerobic effluents which were well flocculated and easier to separate show consistency confirming the conclusion that it was fine solids contributing to the elevated P concentrations in the anaerobic and anoxic samples.

Anaerobic tank: - The pH for line A was at 6.0 (Figure 45) and released PO_4-P in the anaerobic tank from dissolving the calcium phosphates bond (Figure 50);

- For line B which did not have extra PO_4-P from the glucose to release and thus should have been below the feed concentration (Figure 51), but for the analytical problem.

Anoxic tank: both lines described a reduction of soluble P by half, but the concentration however was better or lower with glucose than milk.

The general lower level found in Figure 51 was also explained by the fact that filtration of the samples was easier with glucose than those from milk, and despite the method of analysis, the data collected were more objectives than milk (before the 9th June). Moreover, it was observed that the further the sample was taken down the length of the anaerobic tank, the easier the filtration was. It was clear that fewer solids were mixed in the samples from the aerobic and F.E. tanks and it can be assumed that the soluble P results were representative.

Line (B) glucose is close to or below 1 mg/L WWWTD level before filtration. Line (A) milk has a curve always below 5 mg/L but never showed any results below 1 mg/L. Both aerobic lines described a substantial reduction of soluble phosphorus concentration and line (B) glucose when the standard error of +/- 1 mg/L is taken into account (Section 4.5.3) would meet the WWWTD. For line (A) milk the P was higher to start with, and the reduction in soluble P was actually better.

6.2.3.7.3 Phosphorus content in final effluent

Figure 52: Phosphorus in final effluent in line (A) milk

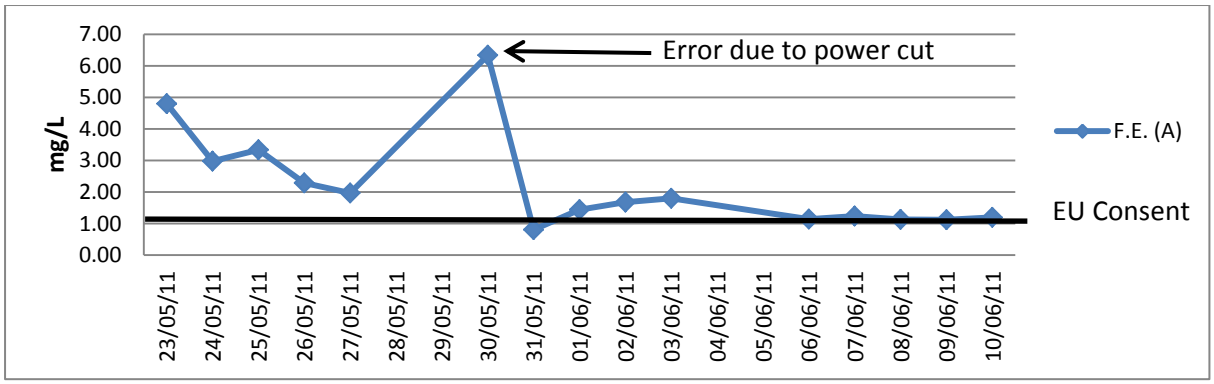


Figure 53: Phosphorus in final effluent in line (B) glucose

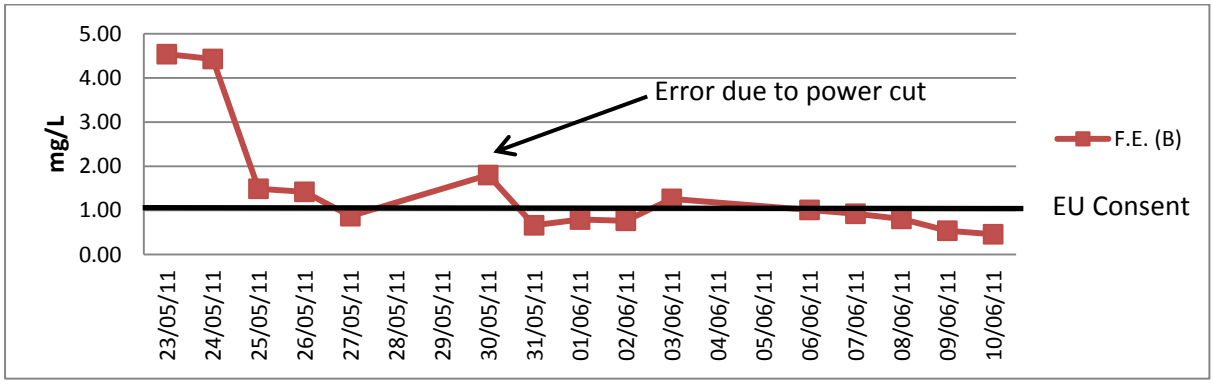


Figure 54: Phosphorus in line (A) milk after nucleation

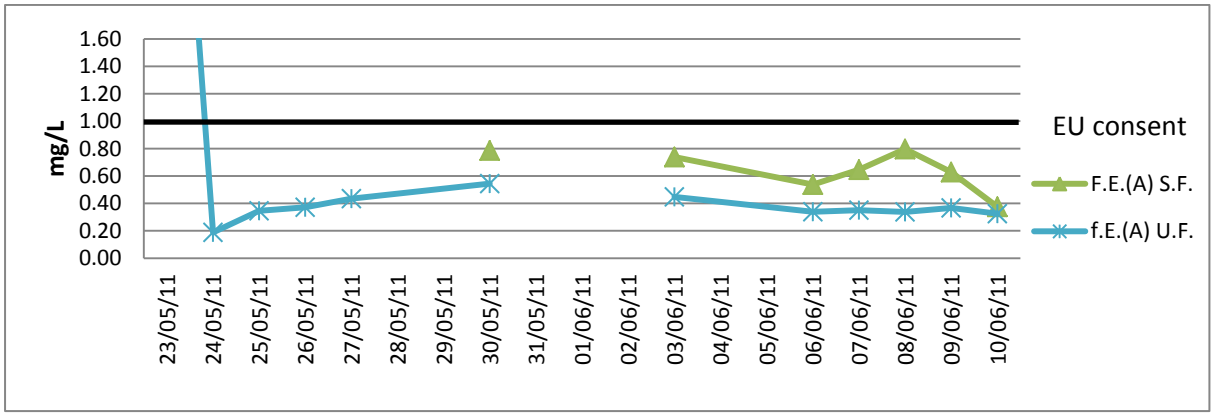
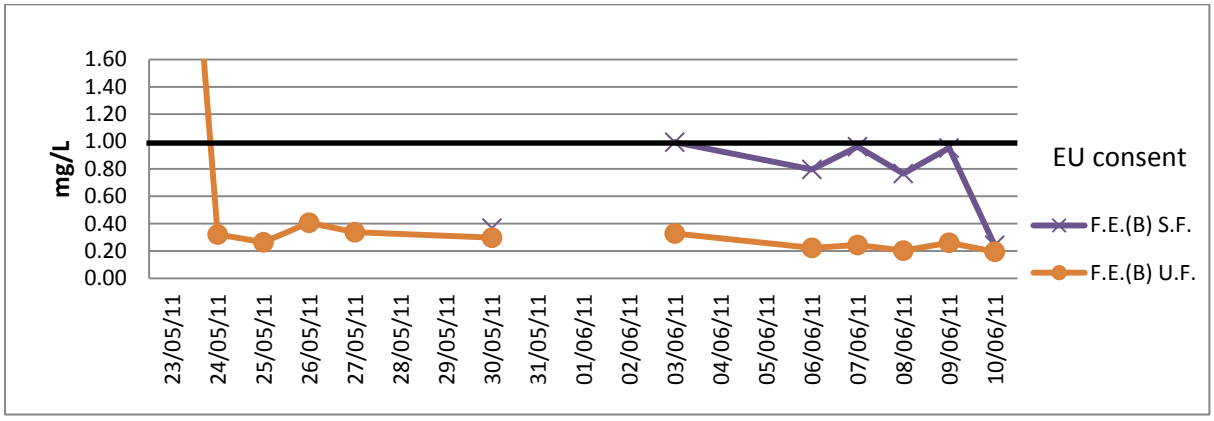


Figure 55: Phosphorus in line (B) glucose after nucleation



The phosphorus content in the final effluent line (A) milk shown in Figure 52 is describing a period of instability. It seems that the system still needed upward of a week more than the acclimation to stabilise and give steady state performance.

Apart from the problem encountered on the 30th the data gave a steady result. The European consent of less than 1 mg/L was often achieved. In the case of line (B) glucose, it never exceeded 2 mg/L after the 2nd day of experiment, and only after a week for line (A) milk.

The phosphorus content in line (B) glucose curve (Figure 53) showed a more rapid adaptation, the soluble phosphorus was either very close to 1 mg/L (twice) or lower (the rest of the time).

The final effluent had passed already through the membrane filtration of 0.4 μm pore size containing only the soluble P concentration. After the process of nucleation, the soluble P concentration has been obtained 2 ways:

1. After a filtration using a 25 ml syringe and an individual filter (11 μm pore size) referred in Figure 54 and Figure 55, as simple filtration (S.F.).

2. After a filtration using a 25 ml syringe and an individual filter (0.45 μm pore size) referred in Figure 54 and Figure 55, as micro-filtration (UF.).

The SF was used to confirm, in the simplest way, that the process of nucleation has created larger particles than 11 μm but the total P still remain below 1 mg/L.

The UF results were constantly below 0.5 mg/L. Chemical nucleation reduced further the soluble P content compared to the F.E. The initial pH (pH_i) rose during the first week from 7.6 to 8.1 for both FE (A) and F.E. (B) and was constant for the last two weeks.

In research led by Mekmene *et al.* (2009), they stated that with an initial Ca/P molar ratio of 1, the pH was a key factor to control the calcium phosphate precipitation. They found that a small, slowly changing pH (up or down) was less efficient at precipitation and not influenced by the Ca/P ratio, whereas a constant pH was strongly influenced by the Ca/P ratio in the aggregation of calcium and phosphate. Despite the fact that their study was carried out at 20°C and our experiments at room temperature (normally lower temperature) and the initial molar ratio Ca/P > 2, the observed results were similar. However, the concentration in soluble P (filtration < 0.4 μm) was lower in line (B) than in line (A). A possible explanation is that the calcium could have been kept easier in sludge line (A) milk (anaerobic, anoxic, aerobic), making it the limiting factor to bond P whereas it was more available in line (B) glucose.

6.2.4 Results and discussion - Phase I: Second Period

This period was done for 3 weeks (from 10/06/2011 to 30/06/2011). **Line A** used the 2.5% milk concentration while **line B** used the glucose mix with equivalent COD. The MLSS from the anaerobic tank were reduced to 1/3rd and membranes replaced by 2 previously cleaned in the laboratory. The purpose was to assess the potential effect for a higher concentration of exogenous carbon source.

Both lines still received 200 ml $CaCl_2$ at 250mM concentration to be injected in two different places, one in the anaerobic tank and the other one in the aerobic tank both at 100ml/24h flow rate.

6.2.4.1 Milk concentration equivalence.

Period II: 2.5% milk concentration: 10g Glucose - 2g Dextrin - 2g Soluble Starch (diluted in 100ml tap water)

6.2.4.2 Collection of sample wastewater in local WWTW

- 125 litres were collected to keep the same characteristics for as many days as possible (Friday 10/06/2011 used from Monday 13/06/2011)

- 125 litres collected (Friday 17/06/2011 used from Monday 20/06/2011)

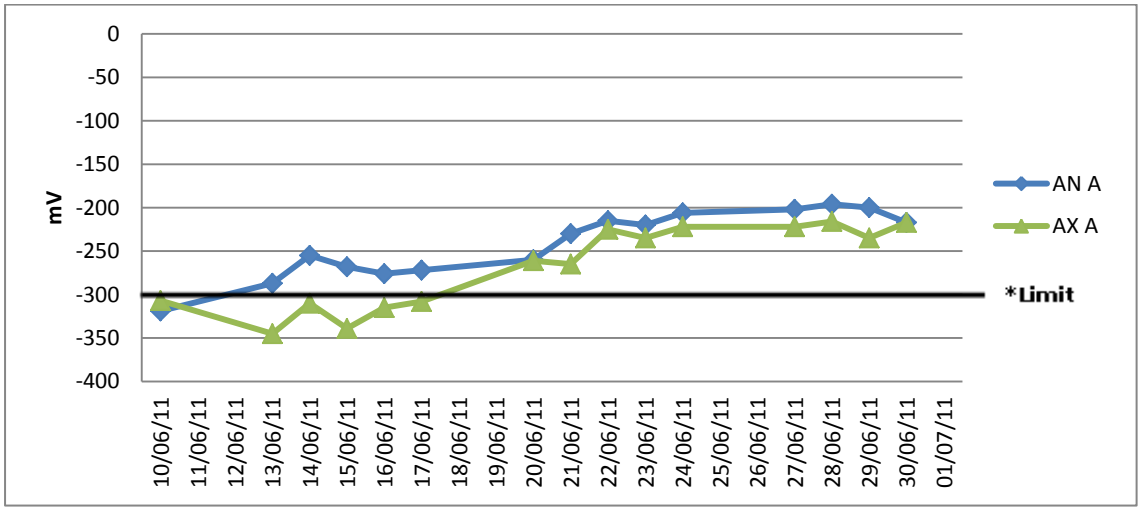
- 125 litres collected (Friday 24/06/2011 used from Monday 27/06/2011)

- Line (A) milk and line (B) glucose are fed separately with these additional nutrients added to the **anaerobic** tank.

6.2.4.3 RedOx from line A and line B

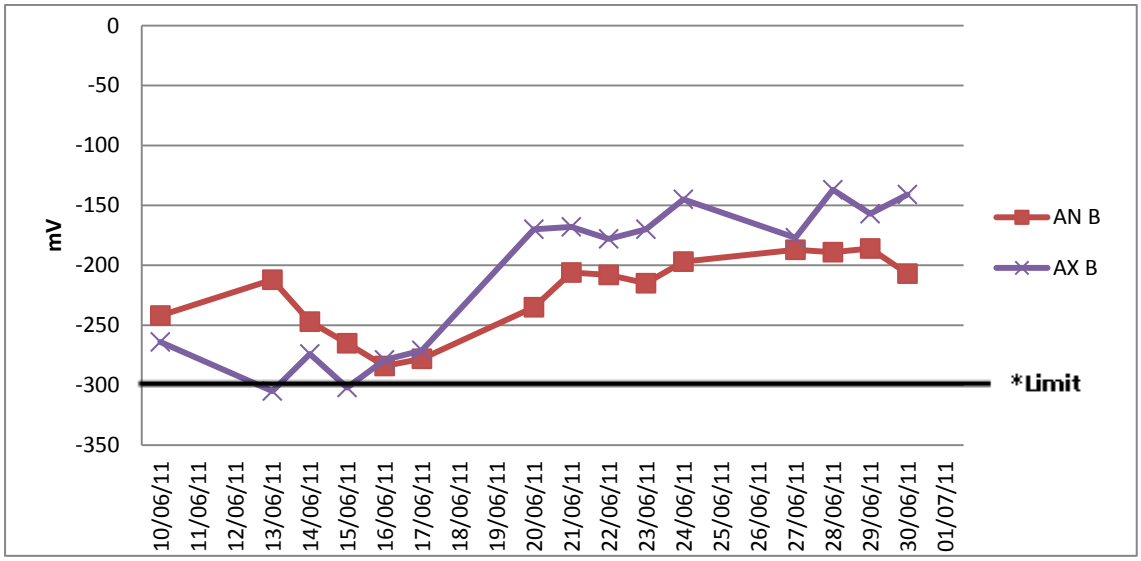
Figure 56 presents the RedOx from anaerobic and anoxic zones from the line (A) milk and Figure 57 the RedOx from anaerobic and anoxic zones from the line (B) glucose.

Figure 56: RedOx line A milk



* Limit is the border between anoxic (0 mV to - 300 mV) and anaerobic (-301mV to - 500 mV) condition.

Figure 57: RedOx line B glucose



* Limit is the border between anoxic (0 mV to - 300 mV) and anaerobic (-301mV to - 500 mV) condition.

By increasing the strength of the wastewater, the start for both lines milk (A) and glucose (B) decreased in RedOx intensity as much in the anaerobic conditions as in the anoxic conditions. However with time the curves increased linearly for line (A) whereas in line (B) the change was more erratic. Anoxic RedOx conditions were achieved in both lines but the anaerobic line (A) curve had a similar pattern to its anoxic curve, but in line (B) the anaerobic zone was mostly in deeper RedOx.

6.2.4.4. pH from line A and line B

The pH of the first Phase second period are presented in the in the Figure 58 and Figure 59.

Figure 58: pH anaerobic - anoxic line (A) milk

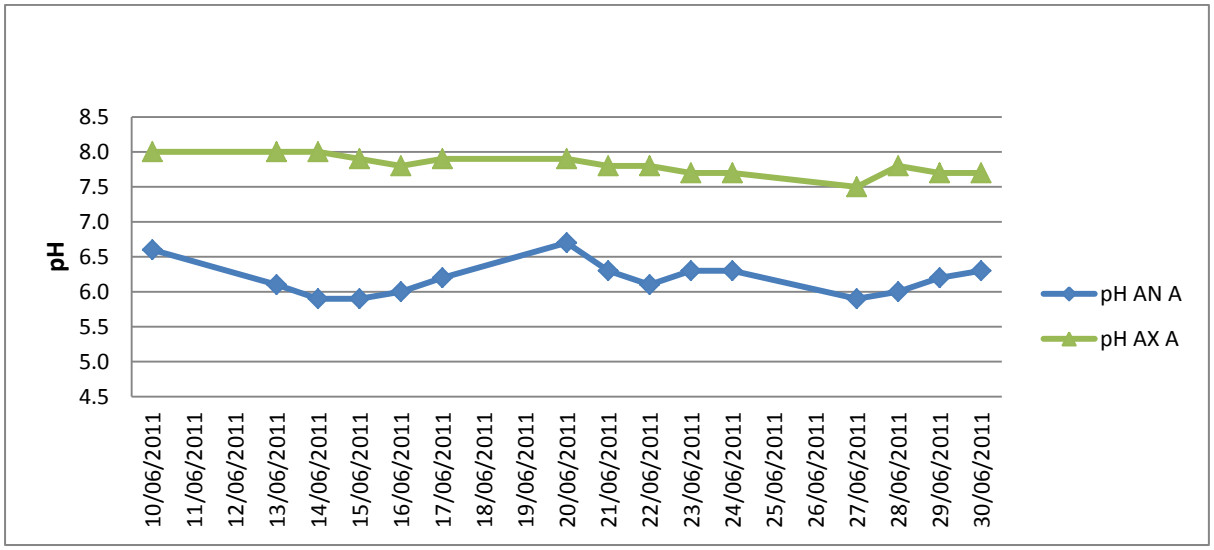
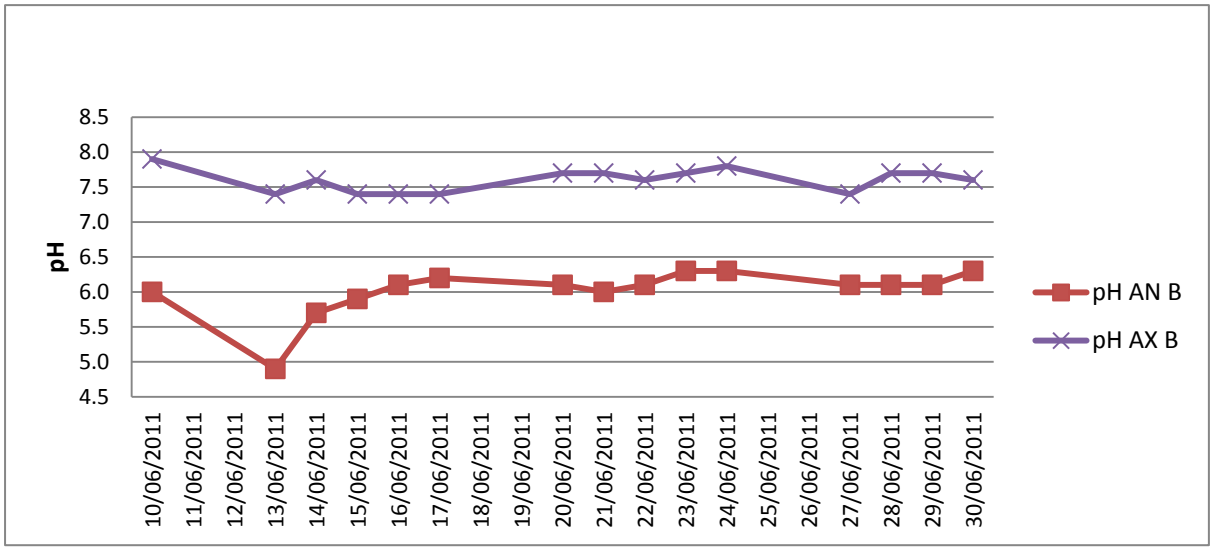


Figure 59: pH anaerobic - anoxic line (B) glucose



The anaerobic pH curves were both in acidic pH range 6.0 – 6.5, but the anaerobic (B) was more acidic. These results suggest organic acids are formed and were consistent with earlier experiment.

The anoxic curves were both alkaline, suggesting the acids were consumed in the anoxic zone and potentially ammonia generated. In this experiment the pH for the anoxic milk line (A) had a decreasing trend, and for line glucose (B), changes were insignificant.

The data from the experiments carried out by Gebremariam *et al.* (2012) using glucose as exogenous carbon suggested that there was phosphorus release by PAOs as long as pH remained above 6.0. Nevertheless, the EBPR deteriorated as the pH declined in their research.The pH curves in the anoxic zones were in both lines neutral and comparable to the previous experiments as suitable for the PAOs.

6.2.4.5 Nitrate concentration and recycle rate.

Recycle rates between the junction anoxic-aerobic tank line (A) milk and line (B) glucose were modified twice, first time on the 14th June and the second time on the 18th, these operations were done to improve the reduction of nitrate which was considered too high in final discharge to be compliant with UWWTD (Figure 60). The adjustment was made by an increase in HRT in the anoxic tank and increase in the recycle pump speed rate. The calculation below was used to finalise the necessary modifications. The details are summarised in Table 22 for the HRT that were applied during the experiments followed by the calculation details.

Table 22: Hydraulic retention time (HRT)

DATE/ZONES	ANOXIC	AEROBIC
14/06/2011	6 hours	3 hours
18/06/2011	7 hours	4 hours
27/06/2011	5 hours	4 hours

Calculation of the HRT shown in Table 22:

Feed flow: Q (L/d)

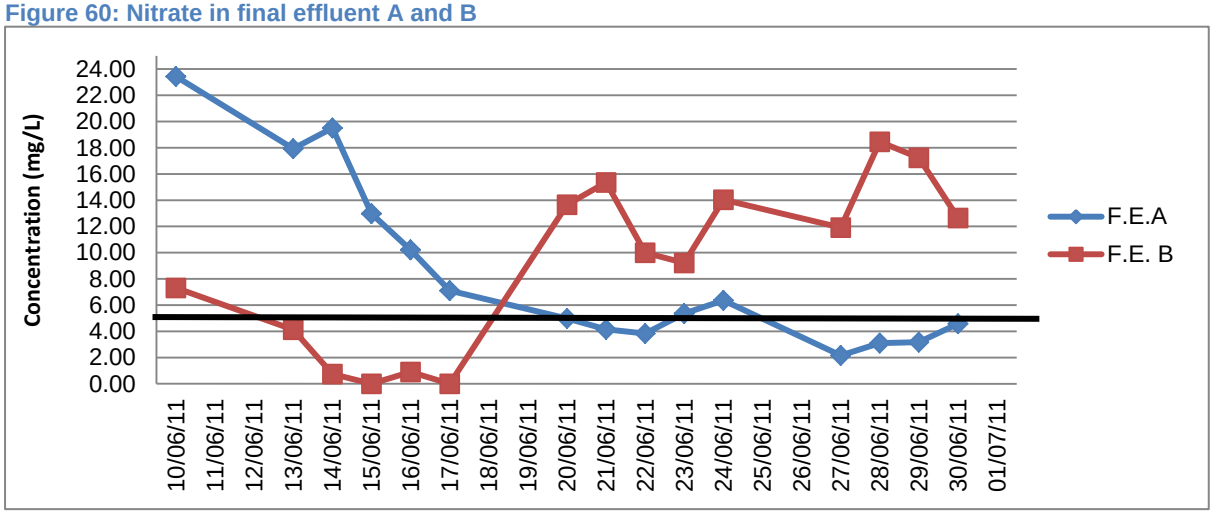
Recycle Flow: (xQ) (L/d)

R1: HRT AX.; R2: HRT AE.

Recycle Ratio (R) = Recycle Rate (L/d) / Feed Flow Rate (L/d)

HRT ($R_{1\ or\ 2}$) = Vol. ($R_{1\ or\ 2}$) / Q (1+R)

Figure 60: Nitrate in final effluent A and B



The consent for nitrate is set at 5 mg/L in the effluent

The nitrate curve in the final effluent in the milk (A) (see Figure 60) shows a progressive reduction of the nitrate concentration irrespective of the increase in anoxic retention. The WWWTD of 5 mg/L target for the milk reactor was achieved most of the time in the second week, and always succeeded in the third week. Thus the HRT of 5 hours for the anoxic tank and the HRT of 4 hours for the aerobic tank (Table 22) achieved the target of nitrate removal in the milk line (A). The pH from anoxic tank was between 7.5 and 8.0 (Figure 58) confirming the conclusions made by Wang *et al.,* (1995) that the denitrifying bacteria work optimally at neutral pH whilst generating some alkalinity (pH between 7.0 and 8.0).

The final effluent nitrate in the curve line (B) glucose (see Figure 60) shows that the system worked efficiently with the HRT configuration with the anoxic tank set at 6 hours and the aerobic tank at 3 hours for the first week. The level as NO_3-N did not go above 5 mg/L and was often 0 mg/L.

In contrast to the milk line (A), with time the nitrate concentration level in line glucose increased and it was concluded that the carbon source (C/N) in the anoxic tank was not sufficient to ensure the reduction of the nitrate. The longer time in the aerobic tank could have led to even more of the available food being consumed and less in the recycle to allow the take up of P in the anoxic zone using NO_3-N, which is energetically less favourable than using O_2 making the metabolism of the microorganisms less efficient. Therefore the conjunction of these two parameters impinged the anoxic bacteria activity. The nitrate concentration suggests that the line (B) glucose is effectively nitrifying but was not able to denitrify. Some researchers have suggested that accumulation of nitrite led to an inhibition of the use of the nitrate during the denitrification (Glass and Silverstein, 1997; 1998a). The pH level was neutral throughout the experiment, so this could be excluded as a reason for decreasing efficiency. The additional aerobic hour was an energy consumer process.

6.2.4.6 pH in aerobic tank and final effluent.

The aerobic and final effluent pH for line (A) and line (B) are represented in Figures 61 and Figure 62 respectively. The pH in line (A) milk did not vary in either the aeration tank or final effluent until the 27th when there was a change, as a result of the shock increase in recycle rate but this was quickly overcome by a new equilibrium.

The pH in line (B) glucose was also sensitive to changes in recycle rate and the effect can more clearly be seen on the 14th, 20th and 27th. It will be interesting to see if there will be an impact on the nucleation later in this section. The small reductions in pH may be as a consequence of inversed activity stimulated by the oxidative potential of the nitrate (the ΔpH is only 0.3).

Figure 61: pH aerobic and F.E. line (A) milk

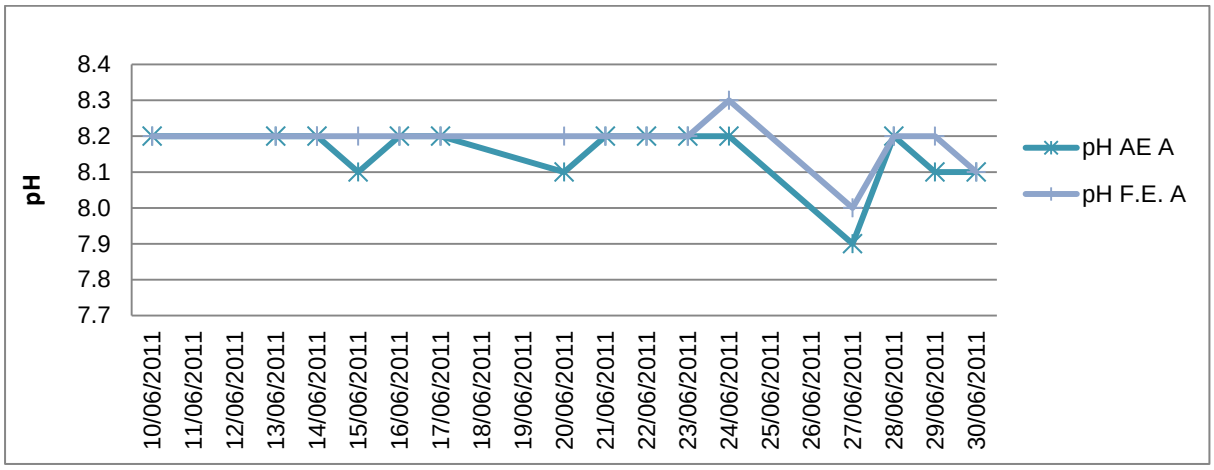
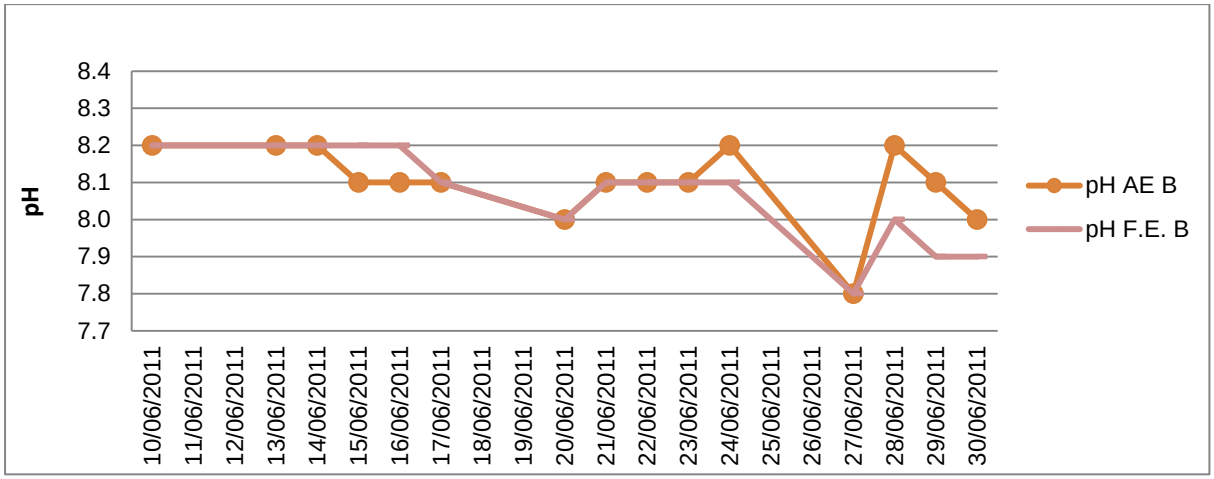


Figure 62: pH aerobic and F.E. line (B) glucose



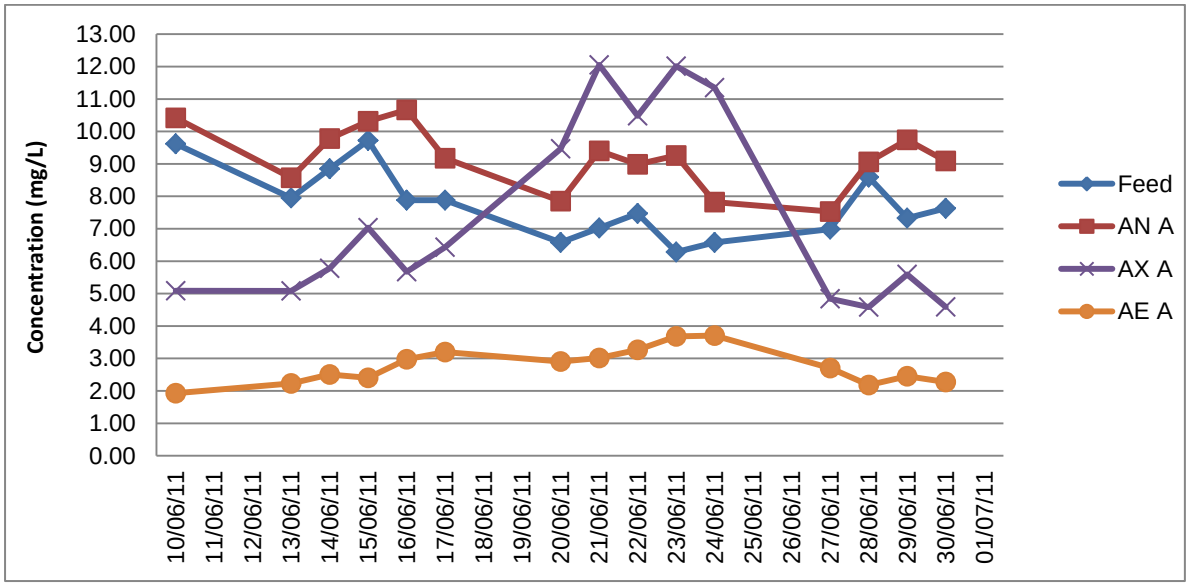
6.2.4.7 Phosphorus

6.2.4.7.1 Introduction

Figure 63 and Figure 64 give the soluble P from feed, anaerobic, anoxic and aerobic zones in lines A and B respectively.

6.2.4.7.2 Phosphorus content before final effluent

Figure 63: Phosphorus concentration in line (A) milk



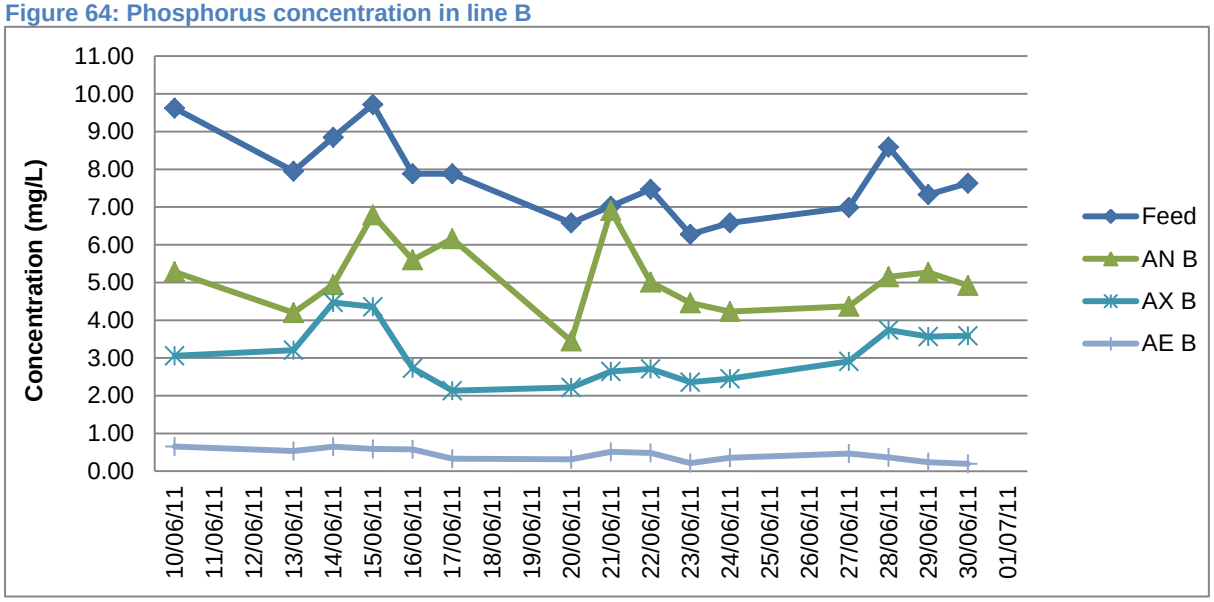
From Figure 63, the anaerobic line (A) soluble P curve is higher than the soluble P in the feed. This follows previous work and was anticipated. The soluble P in the anaerobic zone with the milk line (A) (figure 63) is greater than the sewage and glucose (Figure 64) but this is due to an extra release of P originating from the milk particles. In any event, it could be concluded that the milk chelating agents are unable to bond all the soluble P under anaerobic conditions and at this pH. This observation challenges our starting hypothesis that milk possesses usable chelating agents. The PAOs are excluded as a mechanism for excess soluble P release because there is no recycling loop to involve return PAOs that could bring about PO_4-P release into the MLSS.

The anoxic curve did react to the increase in HRT (see above Table 22, 7 hours). A further release of P has been made by the PAOs as a result of an excessive retention time and low RedOx potential. The observation of the changes from the anoxic curve could suggest different strains of PAOs involvement. This was observed in study made by Hu *et al.,* (2002); Garcia-Usach *et al.,* (2010) who concluded that there were different strains of some PAOs were only able to use oxygen as electron acceptor, but others capable of using either oxygen or nitrate as electron acceptor (DPAOs).

Therefore in this case, the oxygen only PAOs at this HRT and zero dissolved oxygen had to release their P to take up VFAs whereas DPAOs could still take up PO_4-P using NO_3-N as electron acceptor (denitrification was occurring, see Figure 60). However, it is not known if the higher level of PO_4-P release was related to the faster metabolism of PAOs to take up VFAs or from the further degradation of the milk (due to the longer anoxic HRT).

Moreover, the secondary release of PO_4-P by the PAOs as has been hypothesised previously was not possible because this effect, well known to be detrimental for PAOs and thus EBPR efficiency, would result from VFA deficiency (Brown *et al.,* 2011). The C:N:P was not a problem with the 1% milk as had been established in the previous experiment. The pH and RedOx parameters were both ideal to fulfil the anoxic conditions necessary for micro-organisms to achieve an optimal EBPR.

Figure 64: Phosphorus concentration in line B



Analysis of Figure 64 shows there was no P release in the anaerobic line (B) glucose for the same reason stated with line (A) milk, but all the curves were constantly below the feed soluble P. The difference with line (A) milk came from the fact that no additional P was added from external carbon source, and the calcium chloride could have been the deciding factor in chelating soluble P into either colloids or calcium phosphate that has precipitated. The pH was also around 6 (Figure 59).

The anoxic curve does not reflect the change in HRT that were introduced on days 18/06 and 27/06 compared to line (A) milk, the curve was almost flat with a further reduction of soluble P. An explanation for the differences in the pattern from Figure 63 milk and Figure 64 glucose could be the MLSS which are shown in Table 23 from the end of the experience in anoxic tanks. The MLSS was more than 2 times higher in line milk (A) thus the potential of P release was greater.

The second potential reason for the PO_4-P decrease was the higher level of nitrate that provided the DPAOs the possibility to take up available soluble P. The DPAOs and denitrifiers were not capable of reducing the NO_3-N concentration when nitrate concentration in anoxic reactor is surpassing the OHOs denitrification potential, similar results were reported by Musvoto *et al.* (1992). This creates an opportunity to DPAOs to be stimulated and they take up more phosphates, Hu *et al.* (2002) also reported similar results confirming the importance of NO_3^- in the biological phosphorus removal mechanisms.

The third potential reason, the lower MLSS concentration provided more contact opportunity between [Ca^{2+}] and P to create ACP where a partial ηACP had the opportunity to precipitate where the rest remained soluble.

Table 23: MLSS concentration in anaerobic-anoxic-aerobic at the end of the experiment

		Total Solids (g/L)	Ashes (g/L)	Volatiles (g/L)
27/06/2011 *Aerobic*	Line A	3.88	1.00	2.88
	Line B	3.43	1.09	2.34
30/06/2011 *Anaerobic*	Line A	11.10	1.47	9.63
	Line B	9.85	1.18	8.67
30/06/2011 *Anoxic*	Line A	6.23	1.36	4.87
	Line B	2.78	0.92	1.86

The aerobic tank was able to remove most of the soluble P using the combination of the 2 hypothesis, accumulation by the PAOs and the injection of $CaCl_2$. The performance compared to milk line (A) was better in terms of removing soluble P to the lowest concentration but the milk line (A) would constrain more P in total and a further reduction was done at every stage. The soluble P concentration after Ca^{2+} nucleation would confirm this hypothesis if more ACP was created as it would bond the calcium phosphate nuclei in the nucleation tank.

6.2.4.7.3 Phosphorus content in final effluent

The final effluent line (A) milk and line (B) glucose are represented in respectively Figure 65 and Figure 66.

Figure 65: Soluble Phosphorus in Final Effluent Line A

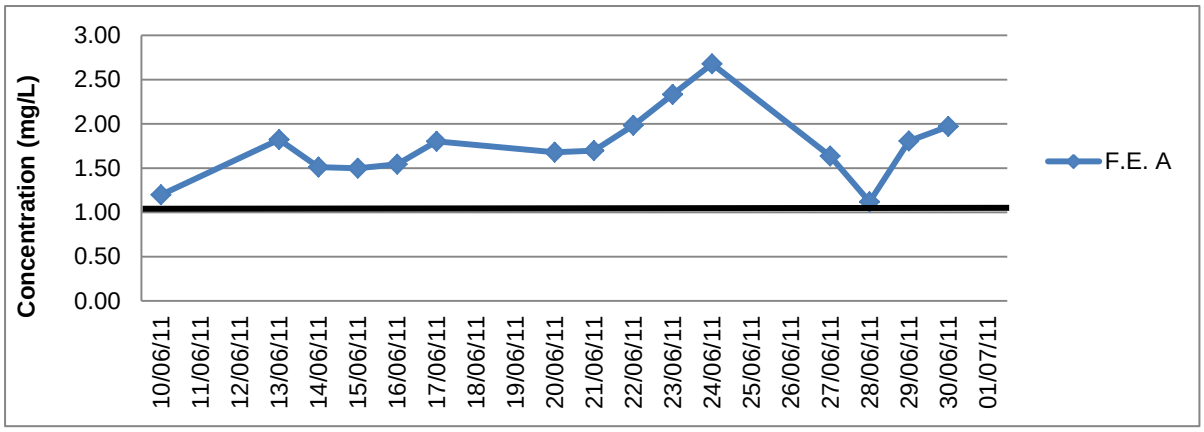
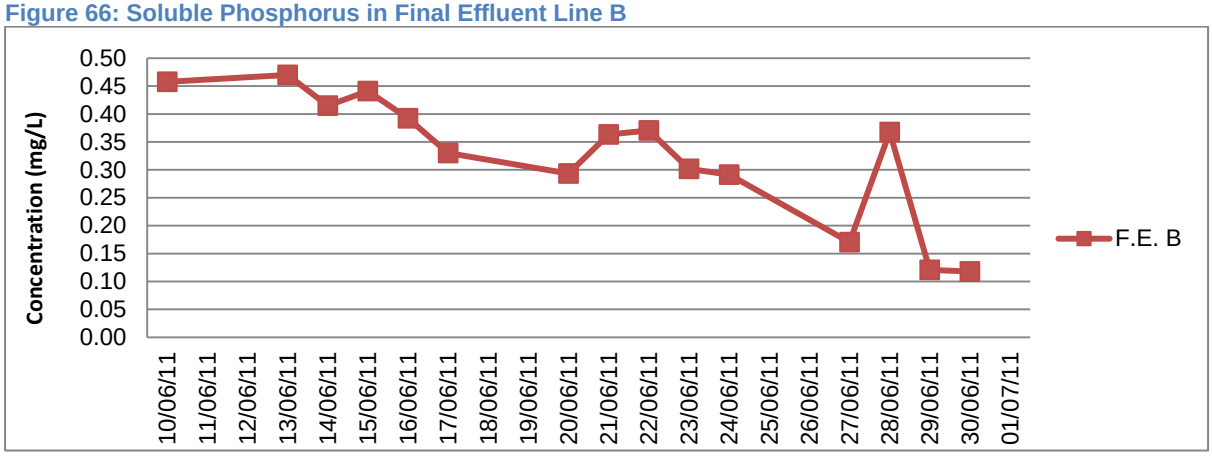


Figure 66: Soluble Phosphorus in Final Effluent Line B



The F.E. (A) shows a further reduction of soluble P. At this stage of the experiments the filtration of the samples anaerobic, anoxic and aerobic supernatant was done with 11 µm pore size and then acid diluted and P stabilised. Therefore, the difference between F.E. and aerobic supernatant was the subsequent membrane filtration at 0.4 µm (membrane pore size) and the 11 µm filter pore size. The Figure 65 shows that the F.E. failed to achieve a discharge lower than 1 mg/L during this experiment. Given the precision of the P test (Section 4.5.3) already referred to however it was concluded there was an expectation the standard could be achieved.

Figure 66 for line (B) glucose was a complete success. The decrease was constant throughout time and never went higher than 0.50 mg/L, therefore the target of less than 1 mg/L was easily and effectively achieved, and did not necessarily need a further treatment

however further treatment was used to compare with the milk line (A), and determine if it was possible to improve the results.

6.2.4.7.4 Phosphorus content after nucleation

Figure 67: Phosphorus in line (A) milk after nucleation

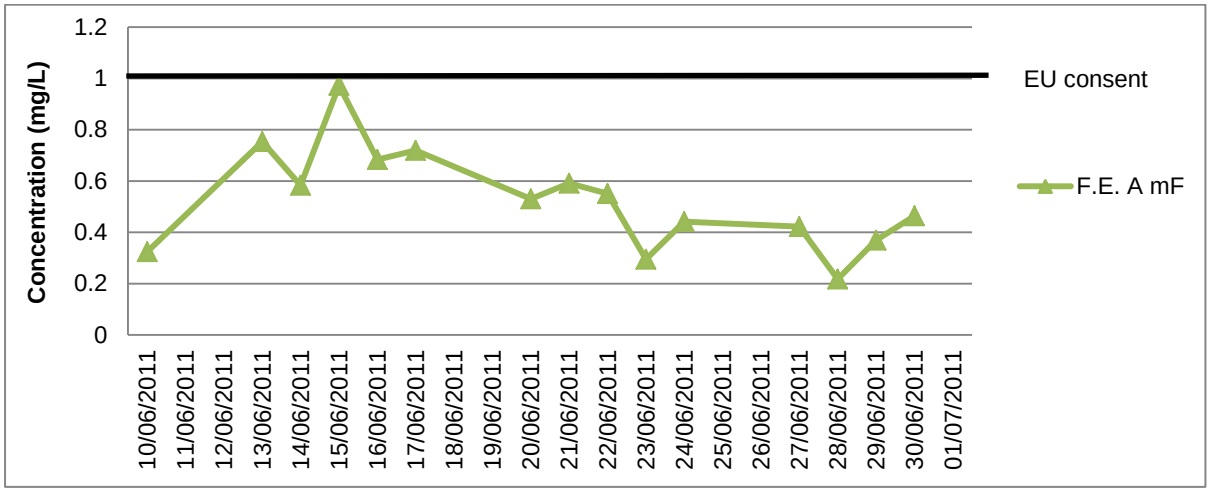
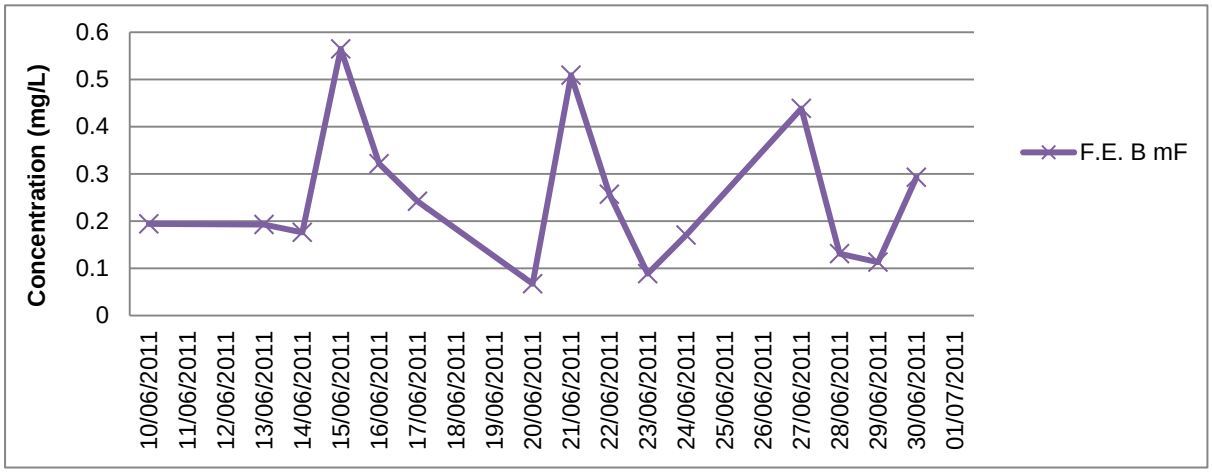


Figure 68: Phosphorus in line (B) glucose after nucleation



Both Figure 67 milk line (A) and Figure 68 glucose line (B) showing the soluble P after nucleation treatment of F.E. with Ca^{2+}. The results were always improved and consistently achieved the discharge standard of less than 1 mg/L for milk line (A). The nucleation of the F.E. glucose (B) provided sometimes “worse results” than the original but in general it has been improved. Table 24 represents the Phase I Period 1, and Table 25 represents the Phase I Period 2, summarising the general performance. The removal from the anaerobic zone was generally higher than from the feed curves due to release, except at the end of period 2.

Table 24: Phase I Period 1

	Average Reduction (%)	Std. Deviation (%)
Feed to F.E. (A)	84.71	5.70
Feed to F.E. (B)	89.39	8.15
Feed to F.E. (AII)	96.58	0.71
Feed to F.E. (BII)	97.43	0.39

Table 25: Phase I Period 2

	Average Reduction (%)	Std. Deviation (%)
Feed to F.E. (A)	76.58	7.56
Feed to F.E. (B)	95.81	1.24
Feed to F.E. (AII)	93.19	2.15
Feed to F.E. (BII)	96.78	1.85
AN (A) to F.E. (A)	80.53	5.52
AN (B) to F.E. (B)	93.49	2.31
AN (A) to F.E. (AII)	92.87	4.76
AN (B) to F.E. (BII)	94.52	3.43

6.2.5 Conclusion and remarks for the Phase I

- The addition of an extra source of carbon did not always lead to a rapid or sufficient anaerobic electronegativity, and the results frequently showed that the RedOx was lower in the anoxic zone. It was worse in the second period of Phase I (where there was 2.5% extra carbon food).

- The pH did provide interesting information about the release of VFAs. The anaerobic mixed liquor pH in line (A) milk (1%) was less acid than in line (B) with an equivalent concentration of glucose at 1% milk whereas anaerobic and anoxic pH were almost similar and less acidic at 2.5% concentration. Several reasons may be proposed to explain the patterns:

    1. A direct uptake of glucose by PAOs demonstrated by Wang *et al.* (2002) that induce an efflux of hydrogen ions that lead to the intracellular acidification described by Ramos *et al.* (1989), and therefore also extracellular acidification demonstrated by Myers *et al.* (2005). Normal synthesis of poly-P creates an uptake of hydrogen ions to restore the pH balance with an acidity detoxification mechanism described by Randall *et al.* (2012).

This is a different mechanism from intracellular acidification that inhibits anaerobic P release and lead to deterioration in the EBPR (Gebremariam *et al.,* 2012). However the same studies are describing an increase in acidification when the fraction of glucose increased in the feed, and yet in our results the 2.5 % glucose described a similar pattern to that found with milk Line (A) at both 1% and 2.5% concentrations.

2. Usually acidogenesis was never induced in research using glucose within the anaerobic zone and since the glucose could not be fermented the PAOs and other microorganisms directly absorbed the glucose as substrate. This concurs with the observations made by Randall *et al.,* (1997) and Akin and Ugurlu (2001). The following period using the 2.5% milk and equivalent glucose mix, on the other hand provided adequate substrate to encourage glucose fermentation and VFA so the PAOs could induce and maintain EBPR. This hypothesis could explain the higher and stable pH and would concur with the observations made by Sudiana *et al.,* (1999); Zengin *et al.,* (2010); Gebremariam *et al.,* (2012).

- As a remark, during Phase I, when there was the extra feeding dosage assessment (Period II, 2.5% extra carbon), it was observed on the walls of line (B) glucose that a thick and dark red layer was formed, mainly in the 3rd chamber (cfr flow chart laboratory system Figure 16). This observation was also found in research made by Gebremariam *et al.*, (2012) as their sludge became more orange colour over time. A smaller but significant layer was also present on the walls of the anoxic zone. In line (A) milk a layer also appeared however this one was dark green in the anaerobic tank. However not analysed, the substances did not affect the process performance.

- The amount of MLSS increased rapidly during the second part of Phase I as a consequence of the high load F:M, creating essentially in the milk line (A) milk a saturation of MLSS in the anaerobic tank, and mixing became difficult. At the end of the third week, the settling 3rd chamber, that was supposed to be filled mainly with liquid in order to provide the supernatant to recycle to the anoxic tank, was full of thick and consolidated solids heavier than in the mixing chamber (1st chamber) where the S.S was 11.10 g/l and the volatile S.S. was 1.47g/l (compared to this 3rd chamber S.S.: which were 46.32 g/L and volatile: 4.99 g/L). The glucose line (B) settled easily and despite a similar organic rate the MLSS had a slower growth. The suspended solids were 9.85 g/L and volatiles were at 1.18 g/L in the first chamber.

The following hypothesis can explain the differences in fermentation and MLSS growth:

a. The proteins and fat within the milk are slower to decompose than sugar (monomers) and thus difficult more to ferment, the constant load was creating an accumulation of substrate in the anaerobic tank. It is believe that the fat was the main substance accumulated.
b. Glucose is often referred in the literature to help the growth of GAOs (Mino *et al.,* 1998; Oehmen *et al.*, 2007) although PAOs can also assimilate glucose directly (see point 2 earlier), they have a slower growth rate and are less competitive. Since the milk is a more complex substance, other microorganisms than PAOs or GAOs can also thrive and grow faster in the MLSS, and thus increase the MLSS significantly.

- From this experiment, the MLSS milk line (A) also performed better in reducing the nitrate concentration but at the expense of PO_4-P removal in the anoxic tank. It was concluded that the level of nitrate was high enough to enable the faster rate of metabolism of the denitrifying bacteria. The OHOs were denitrifying more efficiently and outcompeting DPAOs as a consequence of the higher nitrate RedOx induced, leaving little opportunity to DPAOs to take up soluble P.

  In the glucose line (B) on the other hand the anoxic reactor accumulated nitrate and the concentration increased. The change in HRT influenced the nitrate concentrations and provided corroborating information that excess nitrate had a negative influence on P removal. Thus it may be concluded as the concentration of nitrate was still present at the end of the process that it still needs some further design modifications to achieve good nitrate removal or at least understand its effect on P removal in order to ensure a final N concentration of less than 5 mg/L. Nonetheless, the level of NO_3-N was overcome to give complete phosphorus removal over the whole process.

- For the Phase II, 1% milk was chosen for the experiments because the results from Phase I were conclusive enough, in terms of the 2.5%, feed was too high load and would have required further avoidable operations in the laboratory and likely at full scale such as the removal of excessive sludge and membrane cleaning. In addition, the lower the exogenous carbon that can be used, the better it is for a sustainable treatment measured in terms of sludge disposal, maintenance, oxygen demand and power.

- The glucose mix line (B) was surprisingly given the results from the milk line (A) and the literature very much more efficient to remove the soluble P with the sludge production that was lower. The turbidity was however higher but always less than 1.0 NTU compared to the line (A) milk. Using milk, which was also a contributor to the soluble P concentration, attain the objectives of PO_4-P release of less than 1 mg/L to meet the UWWTD (literature review), the nitrate was also lower than found in the glucose line because the anaerobic and anoxic conditions had lower RedOx.

- The calcium chloride was used in these experiments and injected into two tanks. At the end of this Phase, it was difficult to state which was the more important at removing P. Thus Phase II experiments were devised to try to identify the extent of the benefits from the calcium additions and the optimum point of addition.

6.3 Experiment Phase II

6.3.1 Introduction

Phase II was defined to investigate the impact of the chemical injection zone whilst maintaining a constant exogenous carbon source addition. The experiments were carried out over 16 days to compare where the chemicals would be most efficient to assist the PAOs to remove soluble P. The test period was from 01/07/2011 to 16/07/2011. The experiment was expected to go longer but had to be interrupted by the refurbishment into the laboratory that led to power cuts. Although nitrate reduction was not the main objective of this thesis, it was still important to try to achieve the level of Total-N necessary to comply with the EU targets. From the previous experiments (Section 6.2), the level of nitrate was overcome so that P removal was still compliant.

The modification for the pre-anoxic phase in the laboratory rig was based on the Orbal process (Oehmen *et al.,* 2007). The process with the earlier South African designs was aimed at achieving EBPR with simultaneous nitrification, denitrification (SNDPR). The SNDPR process starts with anaerobic followed by an aerobic reactor (with a D.O. of 0.5 +/- 0.1 mg/L) to create some nitrate followed by anoxic reactor for denitrification and to promote DPAOs using nitrate for P uptake. The experimental line in our experiments had $CaCl_2$ injected into the aerobic tank, and will be referred as the (Line (A-AE)). It also had a modification by adding a new intermediate step between anaerobic tank and anoxic tank to create an external aerobic zone in accordance with the Orbal process. These tests (Phase II) were to evaluate and compare the impact of aeration (less than 1 mg/L from 06/07/11 to 11/07/11 in this tank). The anoxic influent (anaerobic effluent) will have to pass through this extra aerated zone aerated during (30 minutes HRT) before entering the anoxic tank.

This process was to favour the DPAOs in three different ways in accordance with the Orbal theory (Hu *et al.,* 2002):

1. First, it limits the growths of PAOs due to the limited aeration time and intensity (less than 1mg/L of O_2);
2. Second, the nitrification before the anoxic reactor generates extra nitrate externally to increase denitrifying activity;
3. The recycle loop (anoxic-aerobic) could also influence the process in two ways:
    a. If it is increased the recycle created from the aerobic tank could leave residual oxygen that would be detrimental to use nitrate as electron acceptor

b. If too low, the nitrate concentration would be taken up by OHOs rather than DPAOs as they are more efficient.

There was however an incident which was potentially an impediment to the results. On the 13th there was a food shortage for few hours (6 hours) before data collection on the 14th. Therefore the data on the 14th was unreliable and ignored, but the system was still enriched with $CaCl_2$ (the calcium chloride pumps had no automatic security stop after electricity failure)

6.3.2 Lines experiments characteristics

The chemical load was 200 ml/24 hours of 250 mM (milli-molars) $CaCl_2$ speed rate. One line received the discharge in the anaerobic tank and the other line in the aerobic tank.

For this experiment, lines A and B were fed identically: with Settled Domestic Sewage enriched with milk, at 1% concentration.

- Line A was referred as the line with calcium addition in the **aerobic** tank; **(Line (A-AE))**

- Line B was referred as the line with calcium addition in the **anaerobic** tank; **(Line (B-AN))**

After the 6th of July, it was decided to add more phosphorus in the feed because the P level from the natural domestic wastewater sewage was too weak (less than 10 mg/L) to show significant differences and therefore, to interpret the data.

For every 25 litres of settled sewage to be used, fresh calcium phosphate was diluted in 1 litre of R.O. water in a beaker and mix after. The concentration augmentation was made in steps as follows with a mix of di-sodium hydrogen orthophosphate dihydrate and sodium dihydrogen orthophosphate dihydrate:

- First increase: 30 mg/L during the period [07/07/11 – 09/07/11];

- Second increase: 40 mg/L during the period [10/07/11 – 12/07/11];

- Third increase: [50-70] mg/L during the period [13/07/11 – 16/07/11].

- Lines A and B were fed with the same settled sewage with the additional milk enrichment poured separately and directly to the **anaerobic** tanks.

6.3.3 Collection of sample wastewater in local WWTW

The Settled Domestic Sewage was collected identically as in the Phase I from the local wastewater treatment work in Loughborough every Friday in order to work the same wastewater characteristics during the following days of the week.

125 litres were collected and stored in the cold room to preserve as far as was possible the original characteristics (Thursday 30/06/2011 used on Monday 01/07/2011 and Friday 08/07/2011 used on Saturday 09/07/2011).

6.3.4 Initial conditions

Immediately after collecting the data from different tanks on 30th June in the previous experiment in both lines, the MLSSs in line B (previously with glucose mix) were completely removed from all three tanks (anaerobic, anoxic and aerobic). The red photosynthetic growth was completely removed after a thorough cleaning.

The idea was to use acclimatized microorganisms from the first experimental phase by firstly splitting the MLSS from the anaerobic tank line A (previously with milk) and adding equal quantities to each of the new anaerobic tanks lines. Addition of 200ml MLSS from anoxic milk line (A) (Phase I) was also provided respectively to both anaerobic tanks, completed with settled sewage.

Secondly, the MLSS from the anoxic and the aerobic line (A) (Phase I) were also divided equivalently between both lines. Table 26 shows the concentration of MLSS at the end of Phase I, and Table 27 shows the MLSS concentrations at the start of Phase II. In this way the MLSS was already acclimated and data was collected immediately.

Table 26: Final MLSS of Phase I

LINE A	Total Solids (g/L)	Ashes (g/L)	Volatile (g/L)
27/06/2011	**Aerobic**		
	3.88	**1.00**	**2.88**
30/06/2011	**Anaerobic**		
	11.10	**1.47**	**9.63**
30/06/2011	**Anoxic**		
	6.23	**1.36**	**4.87**

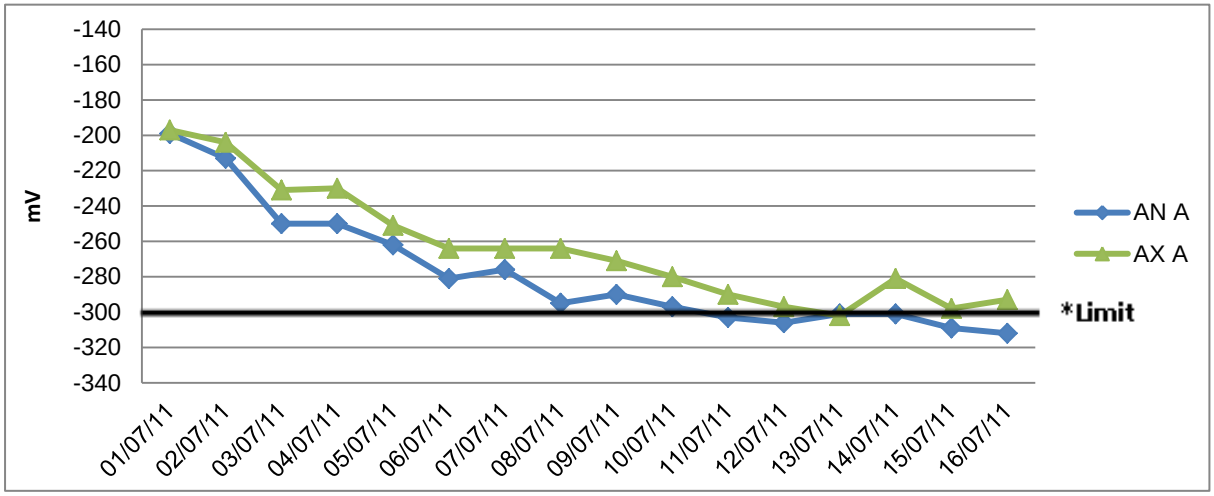
Table 27: Starting Experiment MLSS Concentration (Phase II)

01/07/2011		Total Solids (g/L)	Ashes (g/L)	Volatile (g/L)
Anaerobic	LINE A	**4.34**	**2.54**	**1.80**
	LINE B	**2.67**	**0.81**	**1.86**
Anoxic	LINE A	**3.99**	**1.52**	**2.47**
	LINE B	**3.78**	**1.62**	**2.16**
Aerobic	LINE A	**3.23**	**1.62**	**1.61**
	LINE B	**3.50**	**1.70**	**1.80**

6.3.5 RedOx from line A and line B

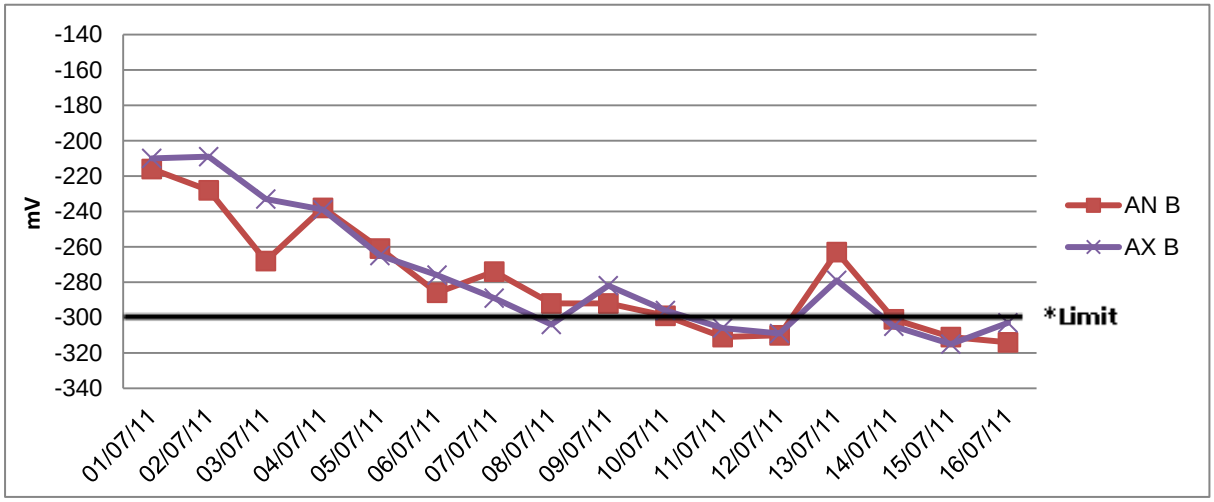
The anoxic RedOx line (A-AE) slightly reacted after the modification by reducing the decrease of its potential compare to the anoxic curve line (B-AN). Figure 69 and Figure 70 show the RedOx curves from anaerobic and anoxic line (A-AE) and line (B-AN) respectively.

Figure 69: RedOx anaerobic and anoxic line (A-AE)



*Limit: below limit, the RedOx is considered as anaerobic condition

Figure 70: RedOx anaerobic and anoxic line (B-AN)



*Limit: below limit, the RedOx is considered as anaerobic condition

The two lines curves are describing a slow decline in RedOx electronegativity at roughly the same rate. It was hypothesised on that good RedOx potential could have had been achieved, because studies made by Barat *et al.* (2006, 2008) stated that important induction of chemical phosphorus precipitation could inhibit the PAOs metabolism.

Nonetheless, this data indicates that the micro-organisms population were not inhibited in the anoxic tank by the addition of the extra calcium (200 ml $CaCl_2$ 250 mM) in the anaerobic tank during this period of time.

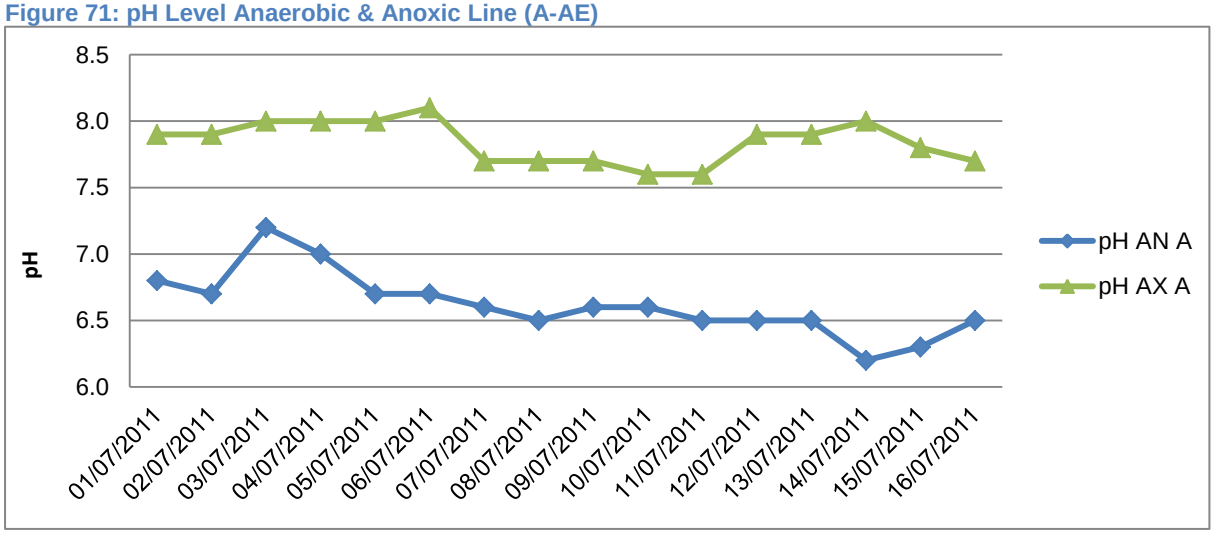
The anoxic conditions were also RedOx electronegative. When the curves Figure 69 and Figure 70 are compared with the curves from RedOx Phase I Period 1 line milk 1% (A), the patterns were identical for both curves, particularly after acclimatization. The curves here started from -200 mV, the RedOx potential decreased gradually in parallel in both the experimental series with and without the extra Ca^{2+} addition. Another observation was that the RedOx potential evolved also in parallel with the increase of MLSS concentration, the experiment started directly after MLSS dilution and thus RedOx declined as the potential oxygen demand and activity from the growing bacteria population increased.

6.3.6 pH from line A and line B

Figure 71 shows the pH in anaerobic and anoxic tank line (A-AE). The curve for the anaerobic zone was in the same range pH 6.5 – 7.0 to the equivalent Phase I Period 1 experiment, although here the curve was closer to pH 6.5 on average.

After the introduction of the intermediate aerated-anoxic zone (Orbal process), there was a step pH reduction that occurred in anoxic tank line (A-AE) (from 07/07 to 11/07), and stopped immediately after the intermediate process was removed, describing a lower stay at level. The pH level in the anoxic zone was relatively stable (around pH 8.0) when the trial Orbal process is not taken in account. Compare to the Phase I Period 1 anoxic experiment, both anoxic experimental data were in the same range 7.5 – 8.0, however in Phase I the curve was slowly increasing to finally stabilise at 8.0 for the last 1/3rd of the time (the last week from 06/06 to 10/06).

Figure 71: pH Level Anaerobic & Anoxic Line (A-AE)

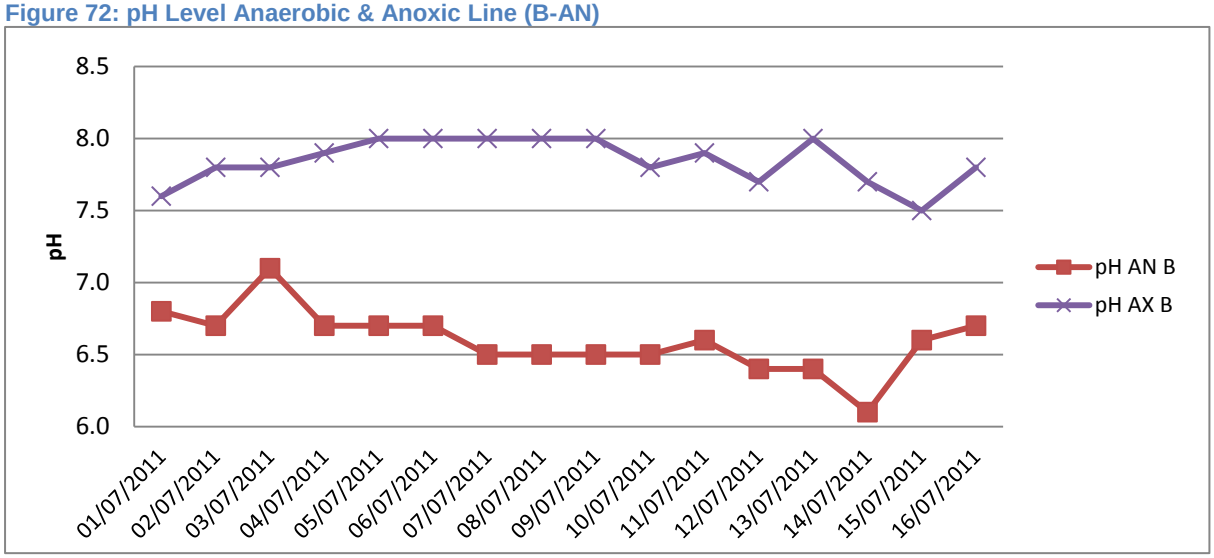


On the 14th, after the power shortage that occurred on the night of 13th, the pump that supplied milk restarted after the power restoration whereas not the peristaltic pump from the settled sewage feed.

The incident created an extra HRT in the anaerobic zone since the plug-flow stopped and a more acidic environment that has been restored after the following 3 days. The come back to the initial state could be the reflexion of the microbial growth rate responding to the extra organic acid available

Figure 72 shows the pH in anaerobic and anoxic tank line (B-AN) (Ca dosing in the anaerobic zone). From the pH analysis, including both Figure 71 and 72, the $CaCl_2$ did not have any effect on the pH level as they appear in the same zones in both the anaerobic and anoxic tanks irrespective of the injection point for the calcium chloride. The introduction into the anaerobic tank line (B-AN), was expected to be a more alkaline when compared to line (A-AE). The effect was opposite from what expected, the pH was always below 7.0 and mostly around the pH 6.5. The pattern of anaerobic curves was almost equal in Figure 71 and in Figure 72 (Phase II) from the Phase I Period 1 (Figure 45).

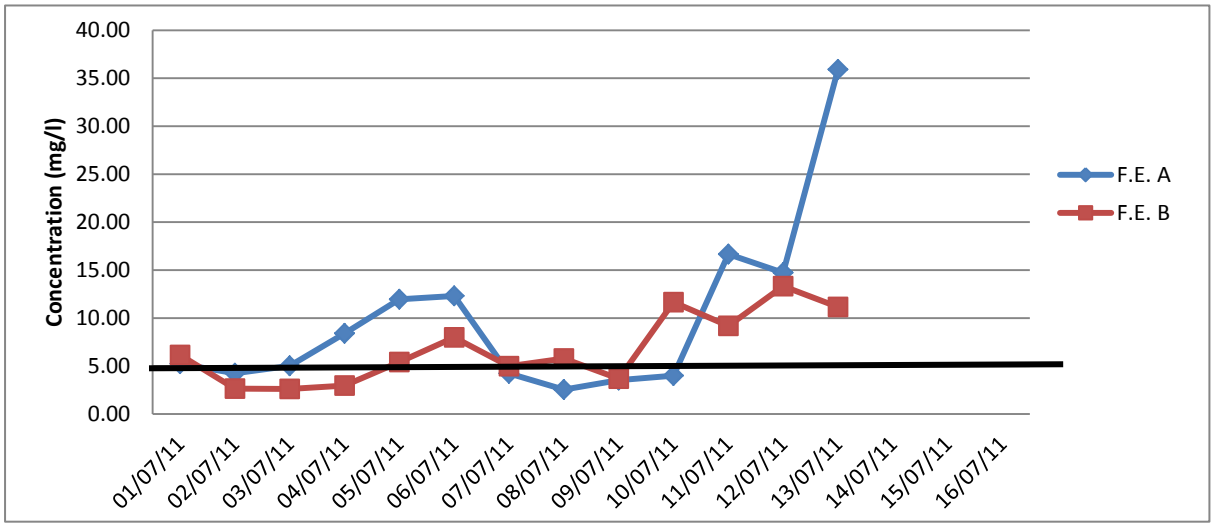
Figure 72: pH Level Anaerobic & Anoxic Line (B-AN)



6.3.7 Nitrate concentration.

Figure 73 below is describing the nitrate in the final effluent from both lines, 5 mg/L is the limit authorised to be discharge in watercourses by the EU environment agencies (UWWTD 1991) after treatment.

Figure 73: Nitrate in Final Effluent



It was intended to reduce the level of nitrate with the introduction of an extra aerated stage (between anaerobic and anoxic tanks) to promote the DPAO metabolism (using nitrate for P uptake) in the anoxic tank by the increase in nitrate concentration. Apparently the process worked successfully in denitrifying as it is shown in Figure 73 but failed to reduce the soluble P since actually the anoxic soluble P was lower before then after this additional aerobic stage test period (Figure 76). This observation suggests that OHOs were capable of extra denitrifying activity and consequently adversely affected the lower denitrification activity of the DPAOs. It is unknown why the nitrate level in the line (A-AE) (aerobic $CaCl_2$ dosage) was in average higher than in line (B-AN) (anaerobic $CaCl_2$ dosage)

The line (B-AN) (anaerobic $CaCl_2$ dosage) promoted the DPAOs because soluble P from the anoxic zone reduced (see Figure 77) compared to the anoxic line (A-AE) (see Figure 76) before and after the artificial P increase on the 06/07. However, the level of nitrate continued to slowly increase with time (Figure 73) after the 10th, suggesting the DPAOs would eventually be overtaken by the ordinary diazotrophs when there is limited nitrate in the tank.

Unfortunately further data collection after the 13th was not possible because of laboratory refurbishment making the Dionex unavailable for N analysis.

Nonetheless, it could be suggested from the data observations that the chemical dosing location was not directly decisive in nutrient removal. The temporary design made it difficult to retain the MLSS in the intermediate stage tank, and after about (6-7 hours) it was noticed that the solids content (MLSS) was lower and consequently would affect the bioreactions.

6.3.8 pH in aerobic tank and final effluent.

The pH in the aerobic zones line (A-AE) and (B-AN) did follow the same pattern as the anoxic zones (Figure 74 and Figure 75). The influence of the pH effluent from the anoxic tank had a consecutive impact on the aerobic pH, but during these experiments (chemical dosing assessment (Phase II)) the pH was expected to have obvious differences to be compared with the previous experiments Phase I Period 1. The pH aerobic curves and final effluent line (B-AN) (Figure 75) was very stable at around pH 8.2 for most of the time, whereas the intermediate aeration process created a change in the pH from the moment it was implemented until its removal of the system. It can be concluded that in normal operation the pH in both lines were ideal for calcium phosphate precipitation (Mekmene *et al.,* 2009) or nucleation (Pouliot *et al.*, 1991), and also PAOs uptake (Oehmen *et al.,* 2005) as they are reported working better in alkaline environments.

Figure 74: pH aerobic and F.E. line (A-AE)

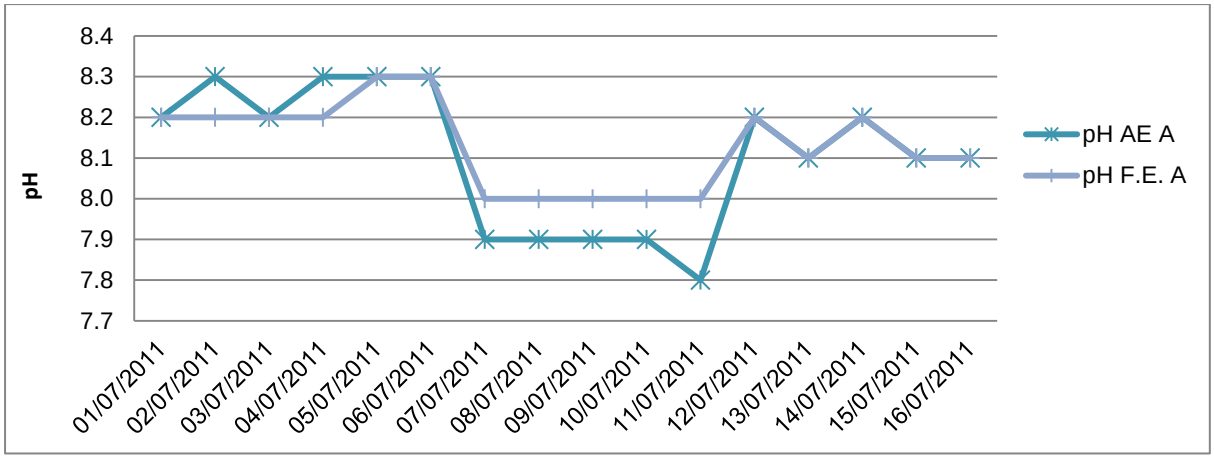
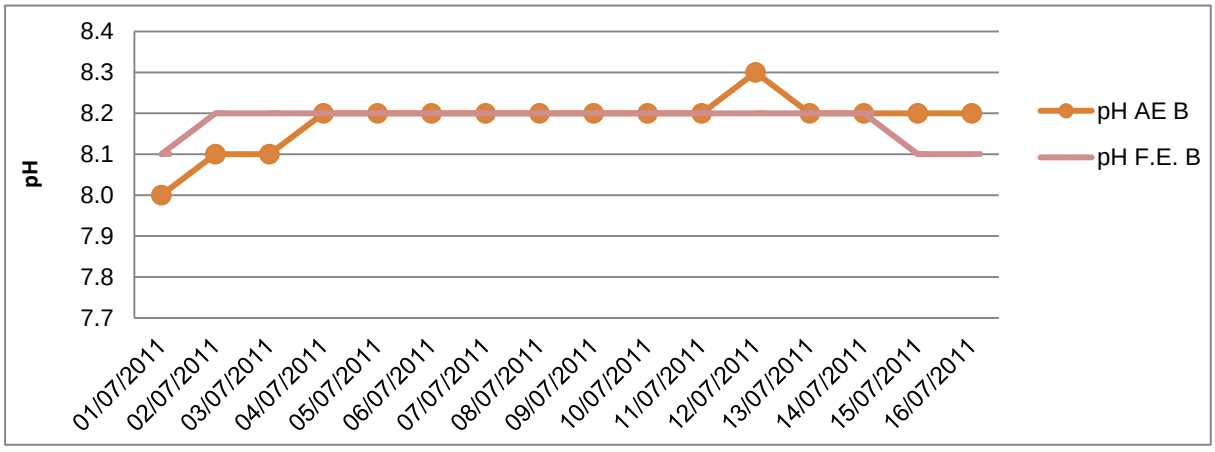


Figure 75: pH aerobic and F.E. line (B-AN)



6.3.9 Phosphorus

6.3.9.1 Introduction

Figures 76 (line A-AE) and Figure 77 (line B-AN), show the soluble P concentrations from the feed, anaerobic, anoxic and aerobic zones. The laboratory refurbishment stopped the analysis of soluble P from the 16th. The artificial increases in P started on the 6th (section 6.3.2) and data collection from the 7th.

The feed P curve was the same for both lines as they were supplied by the same influent tank (as noted in sections 6.3.5 and 6.3.6). From the additions of $CaCl_2$, there were no noticeable differences between the lines for the RedOx and pH. However, it was expected that the soluble P would be affected by chelation with calcium.

Unfortunately, there may be difficulties comparing results Phase I period 1 because of the problems with the filtration methods used for the anaerobic and anoxic supernatants in Phase I, noted in Section 6.2.3.7.

6.3.9.2 Phosphorus content before final effluent

Figure 76: Phosphorus concentration in line (A-AE)

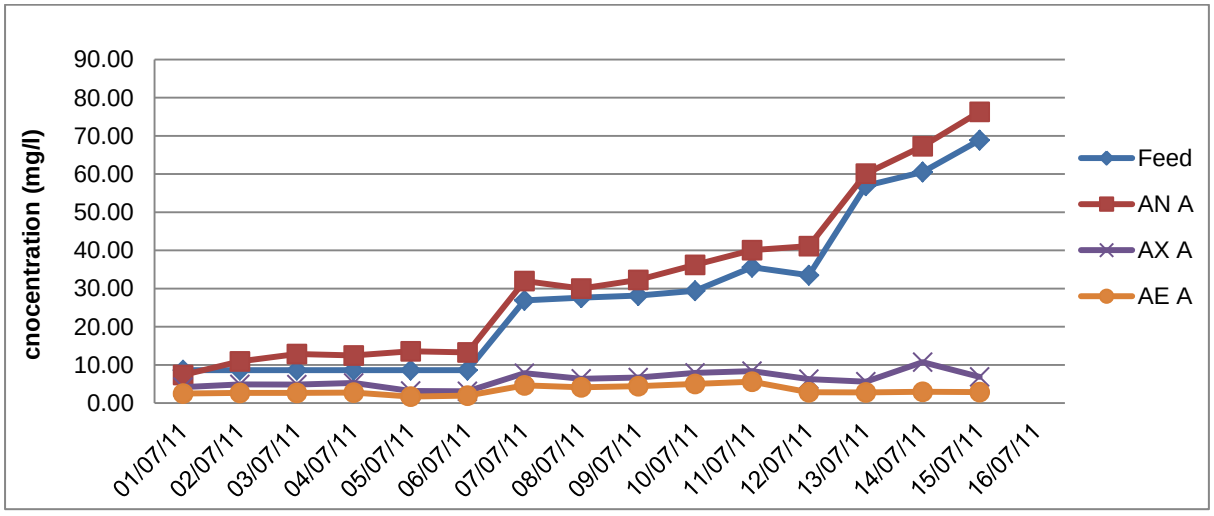
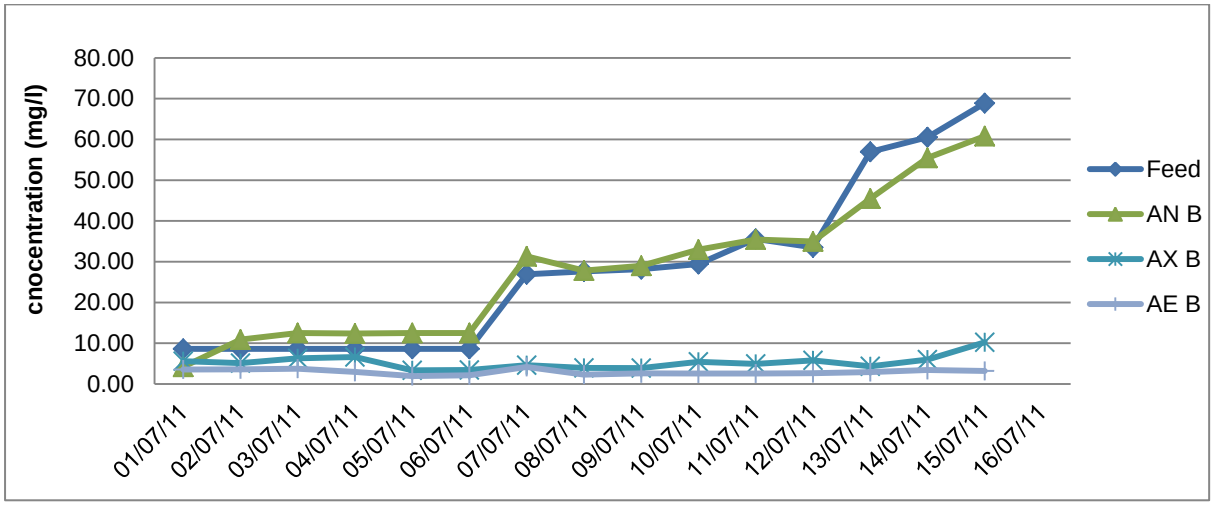


Figure 77: Phosphorus concentration in line (B-AN)



The anaerobic stage curve for line (A-AE) is always slightly above the feed suggesting that more soluble P was released from milk. However, the anaerobic curve in line (B-AN), where the addition of $CaCl_2$ was done in the anaerobic tank was identical. The curves feed and anaerobic actually describe 3 patterns:

1. from 1st to 6th: slightly above;

2. from 7th to 12th: overlapping;

3. from 13th to 16th: slightly below.

This might suggest that the ratio Ca/P could be influential since the dosing to the anaerobic zone is consistent and only soluble P concentration change through step increase. More soluble P could be bonded at the pH and RedOx conditions from the anaerobic dosing zone. Mekmene *et al.* (2009) stated that at pH constant, the precipitation of calcium phosphate was strongly influenced by the Ca/P ratio.

The anoxic zones for both lines described a major decrease in soluble P made more obvious when the concentration of soluble P was made artificially higher on the 7th. Both curves remained stable despite the increase of soluble P discharged from the anaerobic tank. This result from our research concurred with Mekmene *et al.* (2009) conclusions that, when initial Ca/P ratio was greater than 2.00 then most P was removed, and phosphate concentration was the limiting factor.

The results from the previous experiments (Phase I) had already concurred with studies made by the van der Houwen *et al.* (2001, 2003) and their model, where it was stated that calcium phosphate needed to be above super-saturation that will create the minimum driving force conditions to start natural complex Ca-P formation.

It was important however when analysing these results to be reminded that the experiment was made over a relatively short period of time, and that important chemical phosphorus precipitation was reported to negatively impact the PAOs metabolism (Barat *et al.,* 2006, 2008). Thus it was concluded that, a long run experiment was needed to determine if there was a negative impact on the EBPR by changing the availability and speciation of PO_4-P for the bacteria.

The aerobic curves always slightly decreased the soluble P concentration more. This far these experiments confirm that although most calcium phosphate was precipitated in our experiments unlike Barat *et al.,* (2006) experiments, this was not detrimental for biological phosphorus removal, and therefore to the PAOs in the aerobic zone. Further work is needed since the length period of the analysis makes this result inconclusive at this time.

6.3.9.3 Phosphorus content in final effluent

Figure 78 describes the soluble P in F.E. and after nucleation treatment line (A-AE) Ca^{2+} dosed in the aerobic zone. The first point to note is the achievement of the EU P standard. The level of soluble P in the F.E. increased during the Orbal test period and decreased when the test stopped despite a natural increase in the PO_4-P in the feed. Therefore from the F.E. data, it was possible to conclude that the modification to promote the DPAOs metabolism using the Orbal stage did not benefit phosphorus removal, but did achieve further nitrate reduction promoting a range of microbial denitrifyers (Figure 73). The most probable reason for the lack of better P removal originally reported for the Orbal process is that more VFA were consumed in the extra pre-aerobic tank and the PAOs when using oxygen were less capable of competing with this reduction in VFA and P uptake further evidence for this is shown when comparing to F.E curve line (B-AN) (Figure 79). However, the nucleation stage continued to show good soluble P removal performance (relatively less good after the 6^{th}) and compliant with the E.U. standard of less than 1 mg/L.

Figure 79 describes the soluble P in F.E. and after nucleation treatment for the anaerobic dosed line (B-AN). Similarly to the aerobic dosed line, the aerobic soluble P did not attain the concentration of less than 1 mg/L, but was arguably more stable compared to aerobic line (A-AE), mainly because there was not a pre-anoxic intermediate process to disturb the EBPR. The final nucleation treatment, as in the case of the aerobic dose, worked well and achieved easily the authority consent of less than 1 mg/L discharge. As reminder, the final effluent (F.E.) was obtained after the membrane filtration (0.4 µm pore size), and the F.E. after nucleation was the F.E. that has undergone through a further process described in section 4.4.7, where a sample was collected with a syringe and filtered with a disposable filter (0.45 mm pore size) for analysis.

Figure 78: Soluble phosphorus in final effluent and after nucleation line (A-AE)

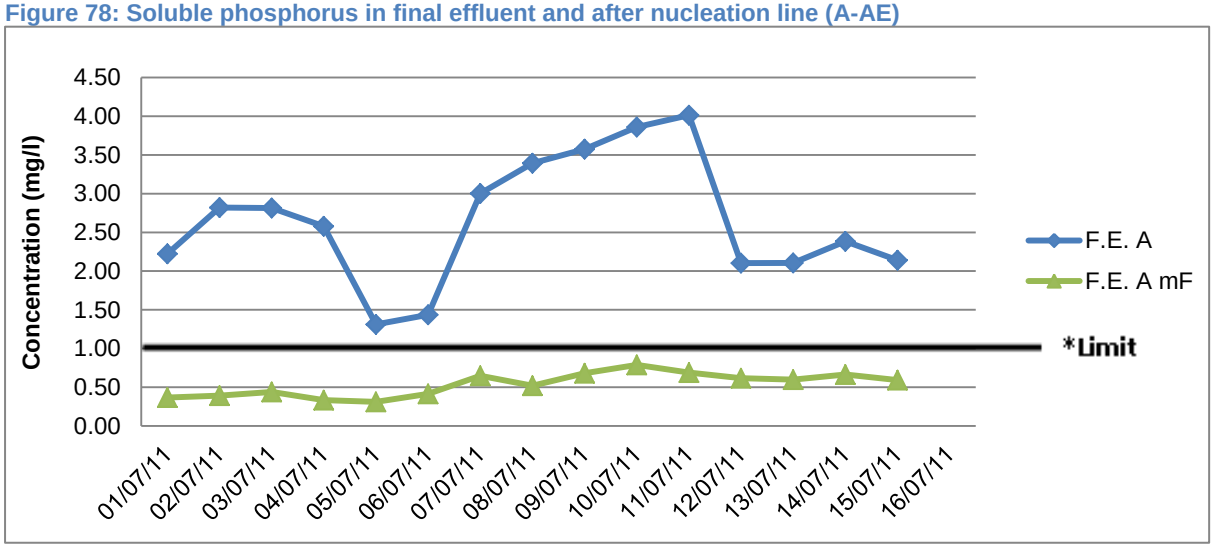


Figure 79: Soluble phosphorus in final effluent and after nucleation line (B-AN)

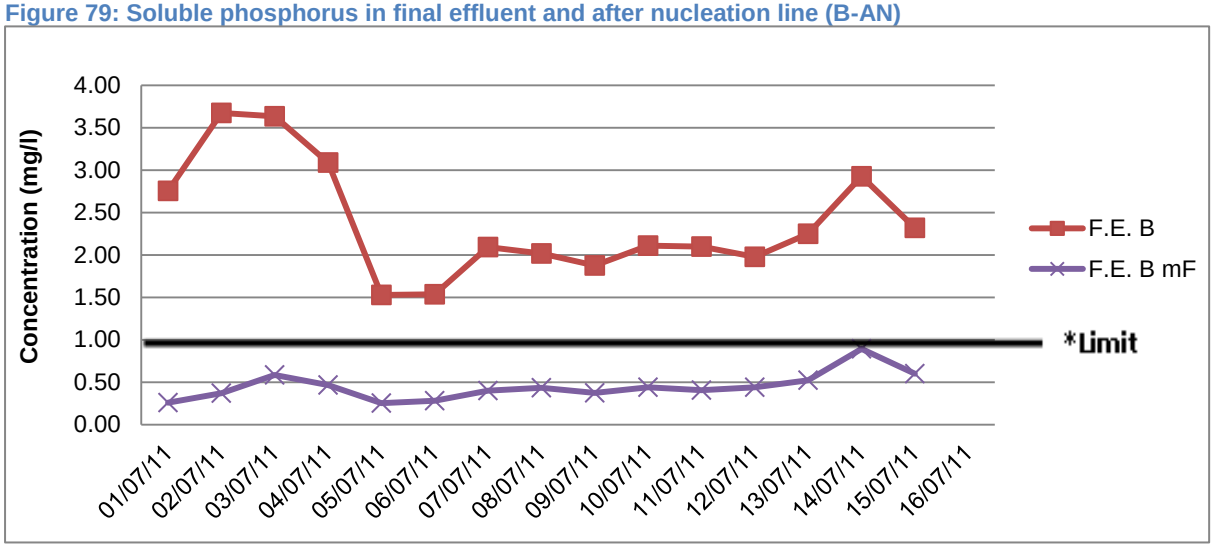


Table 28 is a summary of the overall removal of soluble P efficiency. The results between feed and F.E. are considered as indicative. For the complete removal results, it was important to include the average soluble P reduction measured after the anaerobic zone because of the extra-P added from the milk that was released in the anaerobic tank.

Table 28: Soluble P removal efficiency

	Average Reduction (%)	Std. Deviation (%)
Feed to F.E. (A)	84.63	9.93
Feed to F.E. (B)	83.93	14.12
Feed to F.E. (AII)	97.18	1.39
Feed to F.E. (BII)	97.51	1.73
AN (A) to F.E. (AII)	97.73	1.10
AN (B) to F.E. (BII)	97.92	1.14

6.3.10 Conclusion and remarks for the Phase II

- It was thought that the addition of calcium chloride at different points in the process chain would have led to a difference in phosphate precipitation, providing better and more efficient reduction of soluble P. The results show an identical response from PAOs and calcium induced P reduction irrespective of the dose point in the final effluent.

- When comparing anaerobic and anoxic curves one to each other:

  - Both anaerobic lines A and B followed the same pattern the RedOx reduces slowly until they reach -300 mV, reflecting microbial growth rate;
  - The anoxic RedOx potential curves from both lines for their anaerobic zones followed identical patterns. The pH curves for line (A-AE) and line (B-AN) are both more alkaline in anoxic stage and more acidic in anaerobic stage showing a surplus of organic acids, and confirming the good fermentation of milk.
  - The anoxic zone again in both cases reduced drastically the level of soluble P with residual NO_3^--N, present especially in line (B-AN). It is possible that the system has specialised PAOs to be able to take up P whilst using nitrate as electron acceptor (Oehmen *et al.,* 2007).
  - The micro aerated-anoxic tank did reduce the nitrate concentration further but was not helpful for the soluble P reduction. Two reasons could explain the less efficient P removal, the first was most likely the further depletion of simple substrates and the second the possible presence of nitrite. Nitrite has been noted to inhibit P removal Oehmen *et al.* (2007), as DPAO cannot use nitrite as electron acceptor (Comeau *et al.,* 1987; Hu et al. 2002) nitrite could also have had been also detrimental to denitrification removal as NO_3-N (Glass et al., 2007) for two main reasons,

    - first the mechanism known as accumulation of extracellular nitrite (the bacteria transport the nitrite intermediate out of the cell and later take the extracellular nitrite back into the cell for complete denitrification) which results from the intracellular competition between the reductase (enzyme);
    - and the other is the competition for nitrate among bacteria but also intracellular which is preferred as electron acceptor (Glass and Silverstein, 1998).

Reduction of assimilable substrates availability and then more competition for the DPAOs appeared to be the most impacting cause since the denitrification was effective and not P removal. However, the accumulation of nitrite intermediate is not to be completely excluded in the nitrate increase (Glass *et al.,* 2007).

- When comparing the soluble P from feed to aerobic tank

- The addition of calcium into the anaerobic tank, on average, produced better results compared to dosing into the aerobic stage (Figures 78 and 79). The probable reason could be that the calcium had a longer reaction period whereas the addition of calcium in the aerobic tank, the reaction was in two stages rather than three stages, the anoxic and the aerobic tanks through the recycle loop.
- The experiences showed an influence in the MLSS soluble P concentration level. Below 20 mg/L approximately, the calcium added was not reacting very efficiently to precipitate PO_4-P despite the importance of $CaCl_2$ as shown in the anaerobic curves (Figure 78 and in Figure 79) no difference is shown before 10 mg/L to 15 mg/L. The P removal were more efficient (around 30 mg/L), and the effect was more obvious when soluble P within the anaerobic tank (or the feed) was above 40 mg/L.
- From the experiments in Phase I, the curves of soluble P level in the anoxic and in the aerobic tank remained insensitive to the level of soluble P in the feed. However in Phase I, either period 1 or 2, the level of PO_4-P never exceeded 15 mg/L. The curves in Phase II were almost flat despite the P variations in both lines.
- The aerobic curves followed the anoxic curves, but with a difference of small improvement.
- The soluble P in final effluent was lower than what was initially found in the feed but higher than the 1 mg/L authority consent. The curve in line B (AN-Ca) showed a better, but insignificant result compared to the aerobic dosing line A (AE-Ca).
- As in Phase I, the nucleation of final soluble P demonstrated for both treatment processes, an almost total removal of P. Line A on average above 0.50 mg/L (biased because of the pre-aerated-anoxic test) and for line B below 0.50 mg/L on average.

Other:

- The membranes worked well during this Phase II and did not need extra-care unlike what often found in hard waters (Kim and Yoon, 2010). The membrane line A (AE-Ca) with $CaCl_2$ dosing in the aerobic tank, the membrane (and the membrane chamber) generated granules which were easy to remove and, were the product of calcium dosing whereas the other membrane line B (AN-Ca) produced insignificant calcium granules at the membrane walls. However, at every start of each new Phase of experiments, to avoid problems due to membrane clogging they were changed and cleaned by soaking off line from the experiments.
- Green microalgae growth was observed in the 3rd anaerobic chamber for the first time.

6.4 Experiment Phase III – using glucose mix

6.4.1 Introduction

This phase was carried out during 8 weeks in order to compare the efficiency of using complex with carbohydrate carbons. From Phase I and Phase II it had been determined that a 1% concentration of milk or sugar complex equivalent concentration was enough to achieve a low RedOx potential to help the PAOs to release and then uptake most of the PO_4-P.

This experimental Phase III (Ref. Table 19 in 4.5.3.12) was used to assess if our modified standard EBPR process could with the addition of $CaCl_2$ and the nucleation achieve better results than in Phase I (P1; P2) and in Phase II. The $CaCl_2$ will be assessed to evaluate the extend of its help in the process

Minor improvements to the operation for better reliability were included in Phase III, these were:

- The control line was designated EBPR with settled sewage only to replicate the standard A2O process;
- The addition of milk and glucose mix as organic supplements were therefore assessed at different times (not directly compared), but in the same rig line;
- The soluble P was increased artificially from the beginning. The PO_4-P concentration in this experiment will clearly be higher than that found naturally in most domestic sewage found in municipal WWTW. There were two main reasons for the high concentration test:
    1. Previous experiments had shown a high efficiency of P removal, irrespective of the concentration tested, a performance limit was not encountered in our experiments so far. It was hypothesised that a reduction in milk concentration during Phase III treatment, from 1% to 0.5% would challenge the EBPR results concurring to the limit test of performance.
    2. To accelerate the response of the microbial metabolism and investigate how the culture coped with and maintained biological P removal during a long period of excess $CaPO_4^-$ precipitation.
- Treatment of “natural” soluble P in wastewater will not be assessed in order to try to reach the limit capability of treatment using the process. (The limit being the regulation demand of less than 1 mg/L soluble phosphorus in the final effluent achievement with a maximum of soluble P provided);

- Addition of $CaCl_2$ was done in the anaerobic and aerobic tanks, despite the results of experiment Phase II as it was more efficient to spread the reactions in two tanks;
- The collection and pre-treatment of samples ensured their preservation of the analysis not conducted within 48 hours. The details are described in the Methods Section 4.5 to avoid some of the earlier problems encountered in Phase I;
- The temperature of the experiments during this Phase III was lower as cold winter weather reduced the ambient temperature of the pilot laboratory which was not heated. The range was between 4ºC and 16ºC (when temporarily heated). According to reports from Whang and Park (2006); Lopez-Vazquez *et al.*, (2007), a temperature lower than 20^{o}C favoured PAO over GAO for substrate competition resulting in beneficial EBPR.
- The ICP-OES (Thermo Jarrell Ash) used for the analysis of soluble phosphorus until the 11/11/2011 when the machine was decommissioned and replaced by a new ICP-OES (Shimatzu). During the change over some samples were lost as they became too old for analysis. Further problems were encountered with electrical supply that led to a further loss of samples.

6.4.1.1 Lines experiments characteristics

The experiment with the carbohydrate complex (as shown in point 1 below) was run for the 4 weeks through the 2 lines (from 10/10/2011 to 07/11/2011):

1. Line A: line (AGM) was enriched with 3g glucose, 1.5g dextrin, 1g soluble starch (1% milk concentration equivalent).
2. Line B: line (BCT) was used as a control line but using only the additional $CaCl_2$ to assist the PAO's to remove soluble phosphorus.

The following experiment using milk was run for the 4 weeks through the 2 lines (from 08/11/2011 to 05/12/2011):

1. Line A: line (AM) was enriched with 1% milk concentration until the 14/11 then, from 15/11 to the end of the experiment 0.5% milk concentration;
2. Line B: line (BCT) was used as a control line only with the $CaCl_2$.

The results are presented as in Phase I and II even though some soluble P data is missing because of the two break downs.

Every 24 hours, 200 ml of $CaCl_2$ (250 milli-molars mM) concentration was added to both lines in the anaerobic tanks (100 ml) and in the aerobic tanks (100 ml).

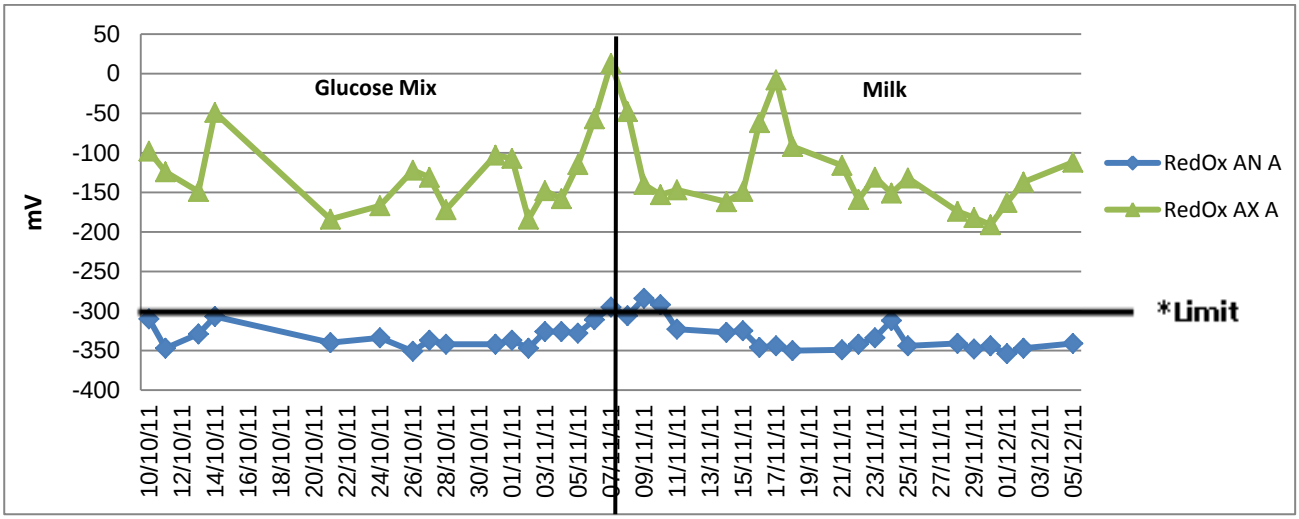
6.4.1.2 Samples collection at Loughborough WWTW.

The collection of fresh wastewater, domestic settled sewage was done on Fridays during the Phase III experiments as before to provide fresh material during the week (from 10/10/2011 to 05/12/2011).

6.4.2 RedOx from line A and line B

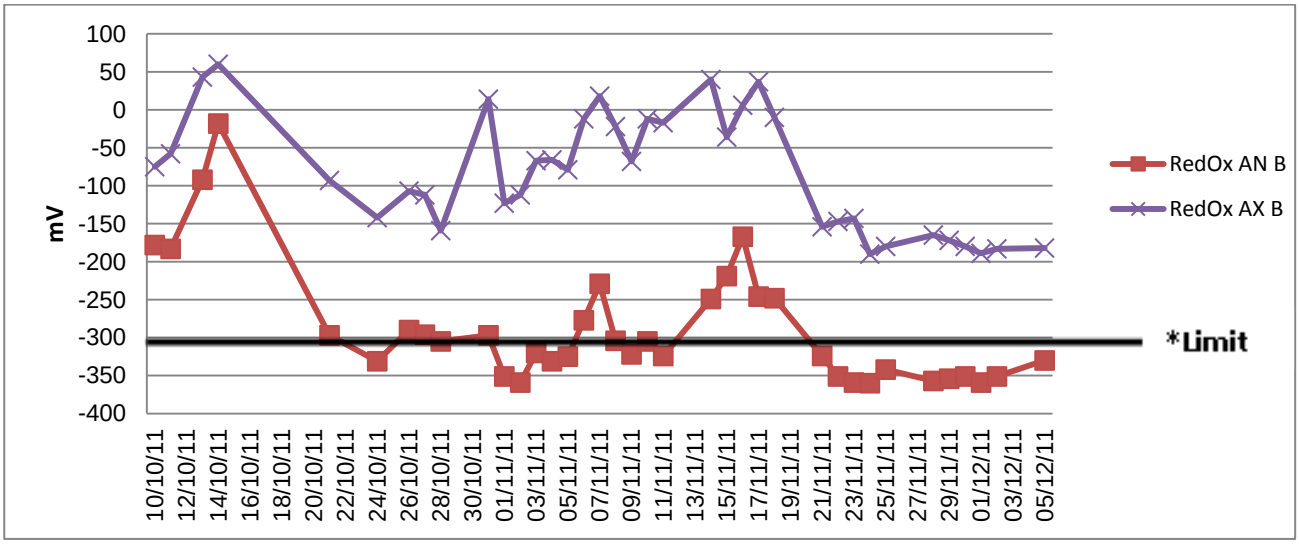
Figure 80 below presents the RedOx from anaerobic and anoxic tanks line (A) glucose-mix (A-GM) and line (A) milk (A-M). Figure 81 shows the control anaerobic and anoxic RedOx line sewage (B-CT).

Figure 80: RedOx line (A) – Glucose mix and Milk.



*Limit: below this limit, the RedOx is considered to be anaerobic

Figure 81: RedOx line (B-CT) – Control line



*Limit: below this limit, the RedOx is considered to be anaerobic

The system has begun without prior acclimation whereas anoxic and anaerobic conditions were immediate for line (A) glucose-mix (A-GM) the control line (B) (B-CT) had more difficulty in achieving negative RedOx. However, during 1 week, from 15th to 20th October both systems were negative and received reduced the $CaCl_2$ concentration initially (100 ml at 250 mM) per day into just the anaerobic tank, the aerobic system had not been started.

Figure 80, shows the anaerobic RedOx (from glucose mix to milk included), and the electronegativity was consistently below - 300 mV throughout the experiments and in pattern with the anoxic RedOx. The different exogenous carbon source could create and maintain complete anaerobic conditions. On the 7th November, the 3rd chamber in the anaerobic tank was emptied, with a syringe, from settled viscous MLSS that accumulated and occupied more than the 4/5th of the volume (this chamber is supposed to be with liquid phase for most of its capacity).

The MLSS increase was important thus a second MLSS reduction was done the 14th November (3rd chamber + MLSS reduction: 2.5 L) (See Table 29)

Three important factors caused the rise on the anoxic curve:

1. On the 7th November, 2.5 L MLSS was replaced with fresh settled sewage (hence curve reached 13 mV) (Table 29);
2. The 15th November, the milk concentration was reduced from 1% to 0.5% (anoxic curve reached -8 mV);
3. The 28/11/2011 after data collection, the MLSS was reduced in the anoxic and aerobic tanks.

After the reduction in milk strength, the response of the anoxic curve was dampened whereas the anaerobic curve showed obvious adaptations even after settled sewage was added to replace the volume of MLSS removed. The reduction of the milk concentration provided less substrates to the anoxic tank and this rapidly reduced the microorganisms activity. The bacteria pool adaptation took almost a week to come back to the RedOx before milk reduction.

The RedOx began to increase in the anoxic line (A-GM) on the 04/11 and this was due to the new growth of microalgae and bacteria accumulation at the bottom of the anaerobic 3rd chamber. The effects are explained in more detail in the discussions and conclusions (section 6.4.2). It is also necessary to point out that for the 24/11 the anaerobic feeding hose was clogged for few hours but had little impact.

As shown in Figure 81, the desired settled sewage RedOx conditions for anaerobic and anoxic environment were less consistently met except for the last two weeks. The anaerobic curve demonstrated mostly the correct RedOx conditions throughout the period, whilst the anoxic curve went periodically close or above zero mV. On the 14th November, the 3rd chamber anaerobic tank (B-CT) as with Line (A) were completely emptied of 1.5 L viscous MLSS in total and partial MLSS withdrawn main chambers to be replaced by settled sewage where the other tanks were untouched (see Table 29).

However, the line anoxic (B-CT) started to deteriorated as it shows a steady increase tendency in RedOx from 28/10 to 15/11 because rain had weakened the settled sewage collected, reduced TOC and the general performance over the period. Thus to overcome the problem it was decided, for a limited time, to boost the influent TOC strength to balance the control line, therefore 1 % milk was added from the 16th to the 20th November (See Anaerobic TOC Annex I).

Increasing the influent strength was the preferred solution as replacing the suspended solids, or changing the MLSS, would not have necessarily worked, and this action, did give extra information corroborating what was happening with the MLSS microorganisms already formed. The comparison between the two lines would have had been biased as the sludge age, impact of $CaCl_2$ among other things would have had been different.

The milk was preferred to glucose-mix to increase COD for two main reasons:

1. The milk is a more complete and complex as a carbon addition to sewage compared to the glucose-mix (less specific);
2. Milk could confirm the hypothesis that it had an effect on improving RedOx of both the anaerobic and anoxic processes.

The combination of removal of unmixed precipitated sludge from the 3rd anaerobic chamber and influent enrichment with exogenous carbon had a rapid beneficial response. This effect suggested therefore that the PAO and denitrifying organisms in the anoxic tank were deprived by VFA availability. A reduction of MLSS in the anaerobic tank reduced competition and was also necessary as it became difficult to mix unlike the anoxic tank were the MLSS was always less than 7g/L.

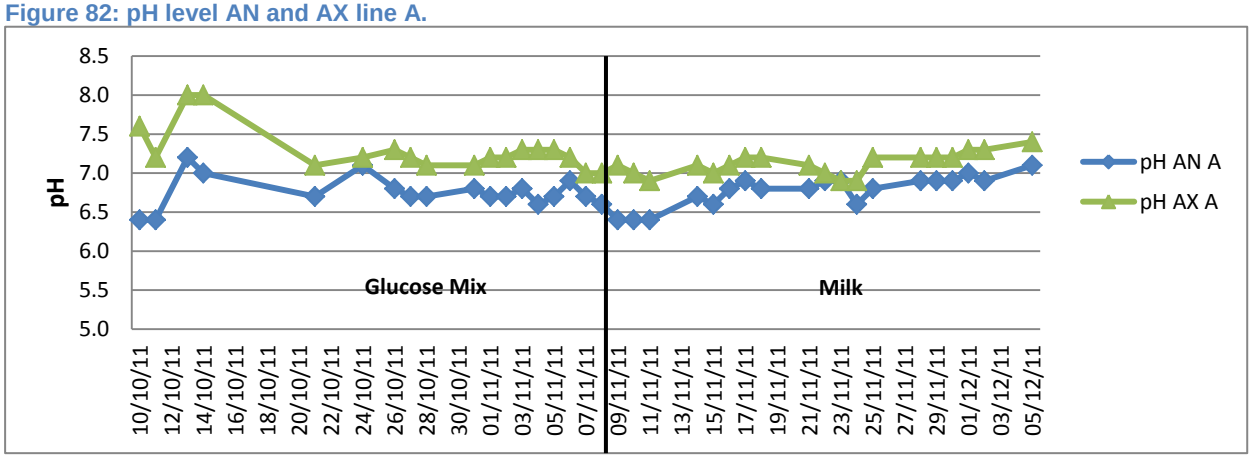
Table 29: MLSS before after anaerobic withdrawn

		Total Solids	Ashes	Volatiles
14/11/2011	AN (A)	**10.47**	**3.85**	**6.62**
Before withdrawn	AN (B)	**11.74**	**7.55**	**4.19**
	AX (A)	**3.41**	**1.95**	**1.46**
	AX (B)	**1.80**	**0.89**	**0.91**
	AE (A)	**5.73**	**3.44**	**2.29**
	AE (B)	**2.19**	**1.24**	**0.95**
14/11/2011	AN (A)	**5.36**	**1.93**	**3.43**
After withdrawn	AN (B)	**2.33**	**1.34**	**0.99**

6.4.3 pH from line A and B

Both the pH and the RedOx (Figure 80, Figure 81) in the anaerobic and anoxic conditions, and in both lines, provide an environment which can support or inhibit PAO's activity. They should also be complementary since VFA are readily formed under anaerobic conditions and directly poured in the anoxic tank. Figure 82 shows the pH level from line (A) (A-GM / A-M) and Figure 83 shows the pH level from line (B-CT).

Figure 82: pH level AN and AX line A.

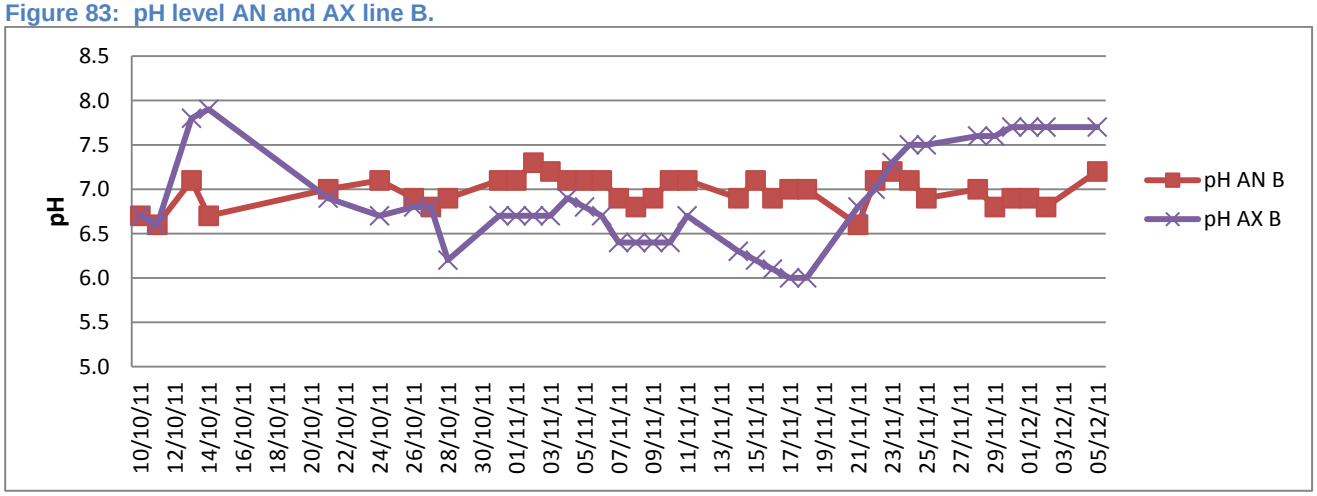


Compared to the Phase I (Period 1 and 2) where the anaerobic curves were acid (pH 6.0 – 6.5) and anoxic curves more alkaline (pH 7.5 – 8.0) Figure 82 above describes curves which are clearly neutral, around pH 7.0, but the anaerobic pH was more acid than the anoxic pH with a tendency to decrease using the glucose mix and a pH that rose back when using milk. The anaerobic pH data suggested that the microorganisms needed about a week to adapt to the transition from glucose-mix to milk as the pH curve had an inflexion after this period. Starting on the 15/11, the reduction in milk concentration did not impact negatively on pH although the pH continued to increase slowly, the RedOx remained constant (Figure 80). However, the anoxic data pH (after the 15/11 reduction in milk) showed a delay and then an adaptation period, whereas the RedOx responded directly (Figure 80).

The anaerobic curve in Figure 83 was below pH 7.0 using the glucose-mix. Based on the research made by Gebremariam *et al.,* (2012) on 4 different glucose/COD ratios (25%, 50%, 75% and glucose only), our research pH data measured in the anaerobic tank gave similar behaviour to their 25% glucose/COD ratio. The pH increase started before the MLSS withdrawals made the 14th (after data collection), as on the 15th the pH registered was lower it was possible to conclude that the pH change was not related to the reduction of MLSS sludge, but to the reduced milk concentration (made the 15th after data collection) where the effects could only be registered as early as the 16th. Thus the slow increase in pH to 7.2 was due to the bacteria acclimation time to the reduced milk substrate to ferment. Consequently less hydrolysed elements and VFAs into the MLSS impacted the anaerobic pH by its increase. This can be supported by the observations made when compared wi7th Phase I Period 2 (2.5% milk concentration) where the pH was between 5.9 and 6.6.

The neutral condition in the anoxic tank was an advantage for PAOs metabolism (Filipe *et al.,* 2001b). Consequently if the nitrate was present and available for the DPAOs, they should be able to take up and reduce the soluble P content in the supernatant compare to the anaerobic tank supernatant.

Figure 83: pH level AN and AX line B.



The anaerobic pH curve, reported in Figure 83 above became steady (until the 05/12) after an acclimation of one week (14th – 20th October) like the RedOx curve. The contrasts between curves appeared when the RedOx started to deteriorate. The milk enrichment (from 16th to 20th) created an inflexion and started to be alkaline that last long after it was finished. The RedOx had a quick response as found in line (A), suggesting that the microorganisms activity was reduced due to the low C/N influent and the pH condition not suitable.

The anoxic pH curve in Figure 83 needed a week of acclimation but the RedOx and the pH were correlated. The acidic environment deteriorated the RedOx whereas neutral and alkaline pH improved the RedOx.

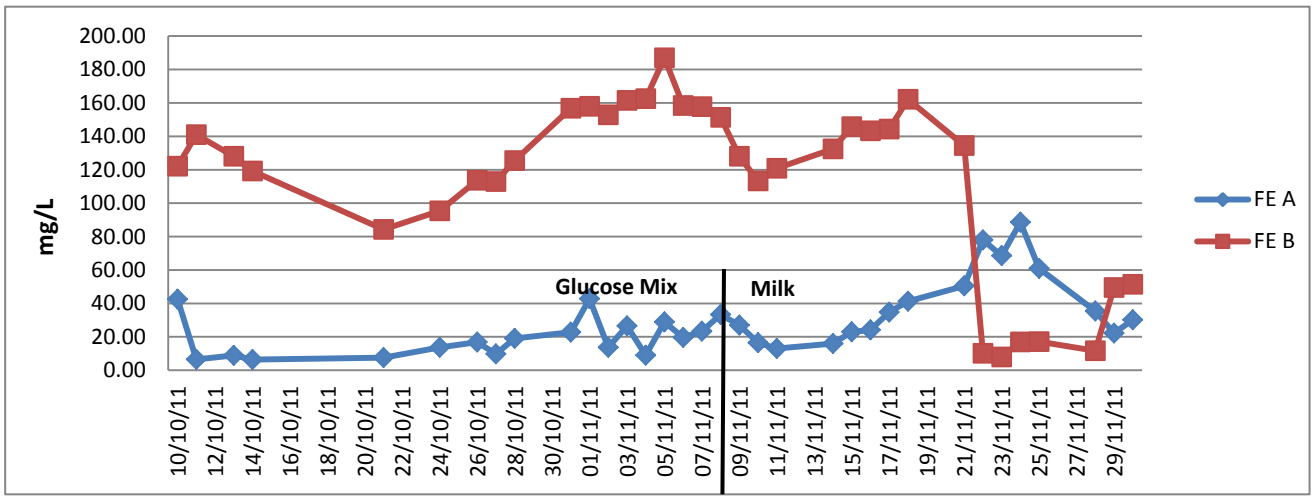
Although the pH increase was concomitant with the reduction of the static microorganism withdrawn and milk addition (see in Figure 81), the acidity could be related to the intracellular pH control, and reducing extracellular pH requires less energy for the transmembrane uptake of organic anions (Smolders *et al.,* 1994; Bond *et al.,* 1999) necessarily true when the substrates are low.

6.4.4 Nitrate concentration in final effluent.

The Figure 84 shows the nitrate concentration in the final effluent. The difference derived from the extra organic matter is obvious between the two lines, and the improvement after the addition of milk to the control line generated a rapid increase in nitrate after the 18th November. Apparently line (A-GM) was performing better but was from a lower total organic nitrogen burden in the feed. Both were far from what the EU discharge consent (less than 5 mg as N), few were below 20 mg/L even in the glucose control. The curve was at higher general level of nitrate than at the beginning, and followed the reducing pH curve pattern (Figure 82). The nitrate concentration could also explain the pH behaviour recorded in Figure 82 for the anoxic curve.

The 1% milk concentration addition to line (A-M) increased the nitrate slowly, but after the MLSS reduction (14th November after collection data) the concentration of milk was also reduced (15th November after data collection), the rate of increase in nitrate was faster from 15/11 to 24/11 (maximum reached was 88.61 mg/L, Figure 84). From the 15th November, conditions were left in steady state and the anaerobic and the anoxic RedOx tanks reduced. The nitrate reduction after the 24th November cannot be explained other than by an increase in denitrifyers activity. No other change was carried out or identified.

Figure 84: Nitrate in final effluent line (A) and line (B-CT).



The regeneration of biomass recovered after the 24th, led to a nitrate reduction until the end of experiment. Wentzel & Ekema (1997); Hu *et al.,* (2002) explained that there were a relatively lower stoichiometric coefficient of efficiency for P uptake by bacteria under anoxic conditions using PHB (and PHV) compared to the aerobic condition. This observation could also be true for the OHO using nitrate (or nitrite) as electron acceptor. It could explain the higher consumption of COD to achieve similar biological nutrient removal in the anoxic reactor compared to aerobic. The reduction from 1% to 0.5% milk, however, did provide enough VFAs for denitrification. It was the sudden change in load that disrupted the steady state.

The nitrate in F.E. (B-CT) (Figure 84) was on average above 120 mg/L (with the peak of 186.99 the 5/11). The high presence of NO_3^- (and probably NO_2^-) created an acid environment, but also with insufficient substrates and biomass became inhibitory to microbial activity of both ordinary heterotrophic organisms (OHOs) and DPAOs (Puig *et al.,* 2007). The combined NO_x^- accumulation and its inhibitory effects have been described by Saito *et al.,* (2004) both nitrite (NO_2^-) and nitrate (NO_3^-) could have reduced the biological nutrient removal efficiency and explained the slow reduction occurred in general activity between 9/11 and 11/11.

After data collection on the 18th the milk was added in the anaerobic tank and the nitrate curve started to reduce after the W.E., on the 21st November. This corroborated the suggestion that the 'rapid' acting denitrifier bacteria were present in tank, but were deprived of sufficient substrates and redox. The milk residual effect lasted until the 28th. The pH increased at the same moment (Figure 83) and the RedOx was reduced for the anoxic and anaerobic curves (Figure 81).

The information generally follows an anticipated pattern for denitrification linked to available substrate and described the interconnections and potential problems of this complex staged system necessary for traditional EBPR. Unfortunately, the nitrate monitoring could not be pursued after the 29th November, because of a problem with the analysis (Dionex) otherwise further alterations of organic additions would have been tried in the control line.

6.4.5 pH in aerobic tank and final effluent.

The pH in aerobic and final effluent are expressed in Figure 85 for line (A) and in Figure 86 for line (B)

Figure 85: pH aerobic and F.E. line (A)

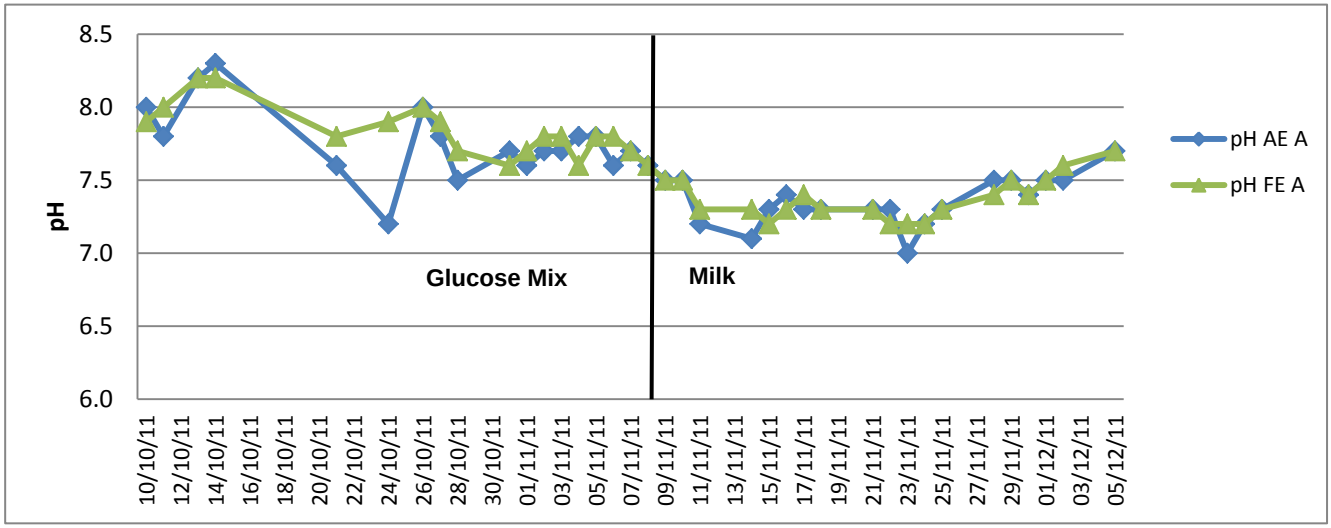
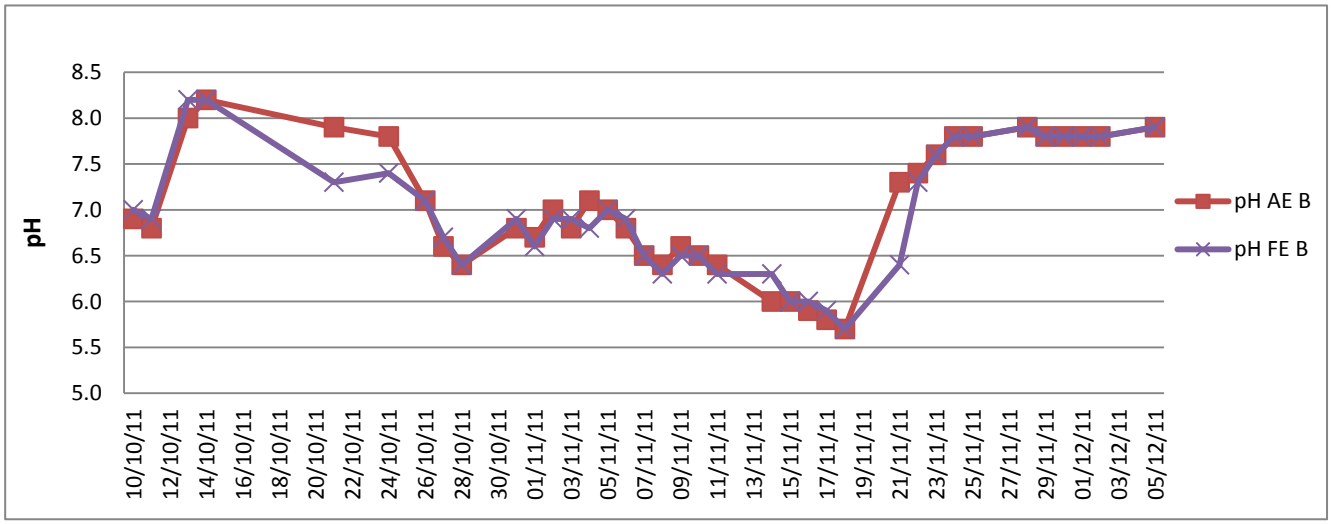


Figure 86: pH aerobic and F.E. line (B-CT)



When the Figure 85 is compared to the pH in the aerobic and final effluent tanks in Phase I Period 1, the pH was less alkaline because of the higher organic load confirmed by the switch from glucose to milk. Similarly in line B (Figure 86) the pH was acidic for some time. Ideally alkalinity is needed for P crystallisation (>7.8) as it was explained in Section 2.6.7.

The transition to milk lowered further the pH, which increased only after changes to the MLSS, reduction in milk dose from 1% to 0.5% (15th November), and improvement to denitrification in the anoxic and aerobic tanks on the 28th November (the pH increased above 7.5).

In Figure 86, the pH for both curves followed the same pattern. The move in the addition of milk to the anaerobic zone after the 18th when RedOx, nitrate concentration and pH in the anoxic and anaerobic zones. This was after two days of exogenous carbon injection (milk 1%), and then the final pH also became more alkaline in response (Figure 86)

Calcium phosphate precipitation increases at stable pH and alkaline pH (Mekmene *et al.,* 2009). Therefore, it was possible to promote P removal by nucleation as long as the pH was alkaline. Moreover, an acidic pH reduces the potential of nucleation compared to the alkaline process (See literature review section 2.5.9.8).

6.4.6 Phosphorus

6.4.6.1 Introduction

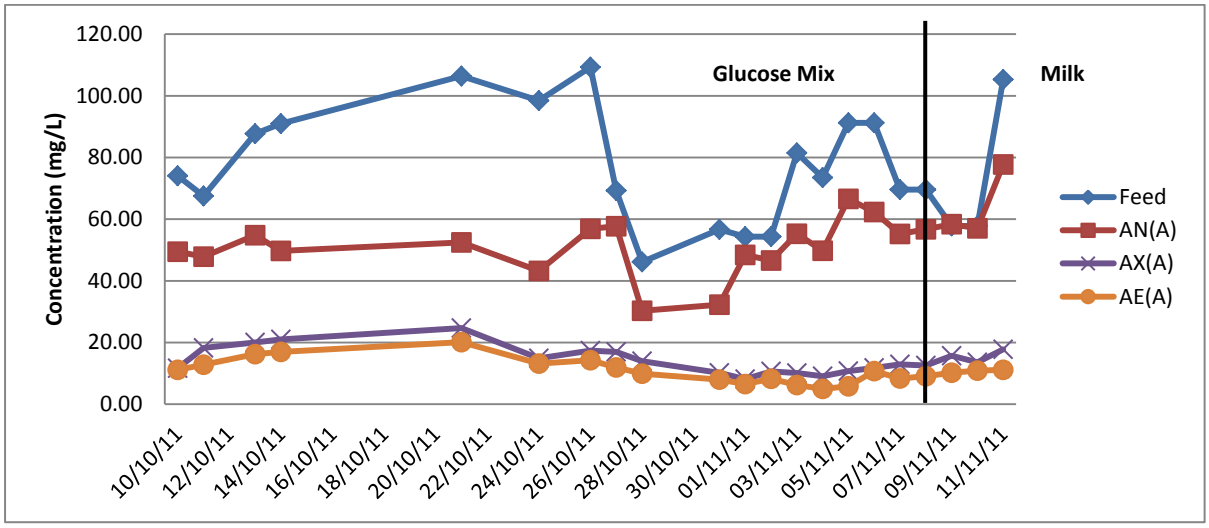
Phosphorus was analysed at every stage, every day (except the W.E.) to assess the release and the uptake of phosphorus. The results have been discussed separately by stage from anaerobic, anoxic, aerobic and final effluent.

The concentration of phosphorus in line (A-GM) (glucose mix supplement) and line (B-CT) (control) for the feed, anaerobic and anoxic are summarised in Figure 87 and Figure 88. The feed for both lines was the same settled sewage with addition of the supplementary phosphorus as described.

The soluble P concentration in the feed during the test was between 40 mg/L and 110 mg/L. These high P concentrations were selected to be able to accelerate the experimental programme through the complexities of the EBPR stages.

6.4.6.2 Phosphorus content before final effluent

Figure 87: Phosphorus concentration in line (A)



The Figure 87 is describing the soluble P content in line (A).

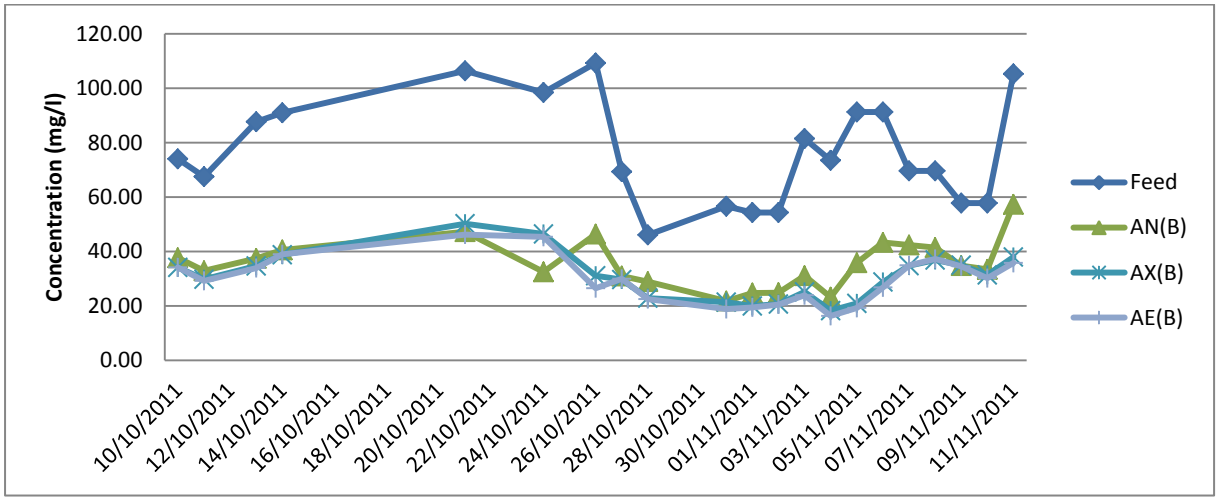
- Generally the anaerobic soluble P curve is below the feed. Normally in EBPR P is released anaerobically (the pH, RedOx and substrates were in the acceptable range for anaerobic bioreactor):

1. In the first part of the curve until 26th included, the larger gap suggests the hypothesis that the saturation induced calcium phosphate precipitation (Ca/P ratio around 1.3 and pH dependent) Carlsson *et al.* (1997). It was noted that saturation was reached when P exceeded 40 mg/L in our experiments. Previous similar observations were found earlier in our study (Section 6.3.9);
2. In the second part of the experiment, from the 27th October, the level of P in the feed was reduced but still created calcium phosphate precipitation. However, on the second occasion when the soluble P feed was increased to 80 mg/L, the difference in ΔP level described between the feed and the anaerobic stage was reduced (for constant pH, Figure 82). The increase in MLSS growth in the system was likely interacting with the [Ca^{2+}] and contributing to a reduction in take up capability of soluble P into the biomass;
3. After switching to milk, the organic P has increased the level of the soluble P but the existing high level of MLSS (10.000 mg) might have now been saturated with P and created soluble amorphous calcium phosphate (ACP). Tis would lead to the similarity between the feed and the anaerobic P.

- The anoxic curve was low in soluble P, suggesting that the DPAOs were taking up the P. The aerobic curve was even (slightly) lower providing a further reduction confirming the general literature that increasing the RedOx leads to P uptake.

It is noticeable that vertically all the reduction of soluble P was done in the aerobic tank. The same behaviour can be noticed for both glucose-mix and milk as exogenous carbon sources. The Figure 88 describes the soluble P content in line (B-CT).

Figure 88: Phosphorus concentration in line (B-CT)



The pattern for the control stream (B) was different at the lower organic load and with the favourable conditions in the anaerobic tank for the PAO bacteria (pH and RedOx). There was an apparent absence of demarcation between the anaerobic curve and both anoxic and aerobic curves.

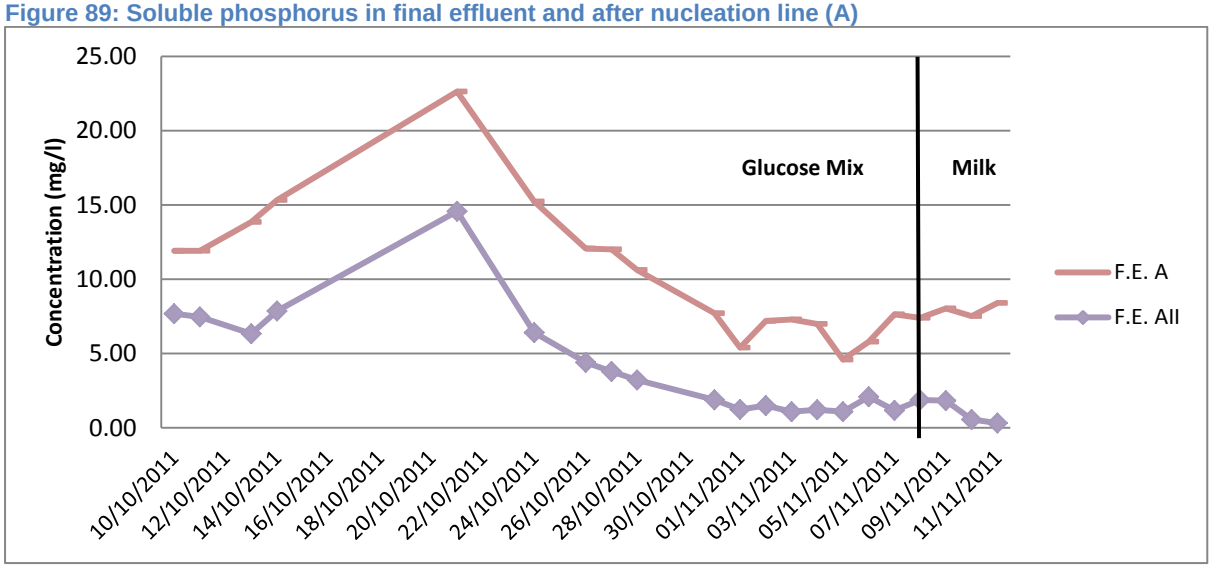
The P take up was not as good as the test Line (A), the probable reason were:

1. Insufficient VFA to promote both nitrate reduction and the DPAOs to take up P (Figure 84);
2. The pH was too high and did not help to promote calcium phosphate complex formation (Figure 83 and Figure 86);

However, the reduction of soluble P in both test and control are more likely to be due to the combination of the addition of $CaCl_2$ to the anaerobic tank and partly the concentration of AOM in all the tanks. The changes in feed P on the 26th October and also on the 2nd November are reflected in the process streams but not to the same extent suggesting buffering from the large amount of biomass.

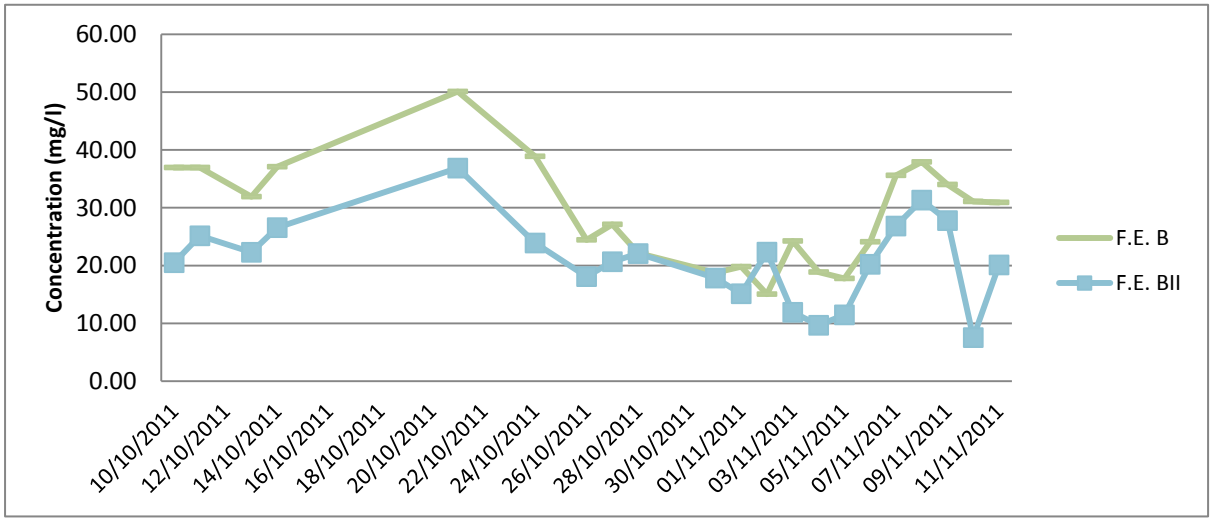
6.4.6.3 Phosphorus content in final effluent

Figure 89: Soluble phosphorus in final effluent and after nucleation line (A)



F.E. A is the final effluent obtained after membrane filtration; F.E. A II: is the treatment of F.E A after nucleation and micro-filtration

Figure 90: Soluble phosphorus in final effluent and after nucleation line (B-CT)



F.E. B is the final effluent obtained after membrane filtration; F.E. B II: is the treatment of F.E A after nucleation and micro-filtration.

From line (A) in Figure 89, the soluble P in the final effluent improves during the experiment, when a period for acclimation is accounted for. The mean soluble P is below 5 mg/L and in general less than 2 mg/L, but more than 1 mg/L (EU consent). Switching from glucose to milk, the level dropped to less than 1mg/L the 10th and the 11th. In contrast, the control without extra carbon but just calcium additions (Figure 90) describes these results line (B-CT), confirming the contribution of PAOs compared to just precipitation.

The results also corroborate that in alkaline pH improves nucleation and precipitation (Song *et al.* (2002) and Mekmene *et al.* (2009)). Figure 90, 85 and 86 show that the ΔP was not as good at more acidic pH.

6.4.6.4 Conclusion and remarks

Neither line consistently met the EU nutrients discharge standard. Line (A-GM) (glucose) gave better removal than line (B-CT), but although line (A-M) (milk) data provided promising good results, they were too few to confirm and state any conclusions.

From the data for line (B-CT), the data suggest that providing a source of readily biodegradable carbon to the system would improve the quality of nutrient removal. The biomass with the support of the $CaCl_2$ (calcium base coagulant) improved the results. Adding the $CaCl_2$ is a complementary aid but not sufficient if it is used alone without enough COD/P/N (see results lines B in Phase III as control system).

It was concluded from both lines that the data when the soluble P in the final effluent was relatively high and in alkaline conditions, the differences after nucleation could reduce P up to10 mg/L. However, when the same final effluent was below 10 mg/L (Figure 89), the response was not linear although close to 0 mg/L could be reached. It could be that there were insufficient solids present to promote agglomeration. As was repeatedly seen in the previous experiments the membrane did not reduce the soluble phosphorus further from the aerobic tank to the final effluent.

Thus the overall conclusion was that line B did not have enough substrate to perform the EBPR and that industrial food wastes would benefit P removal. Table 30 expresses in percentage the reduction of soluble P from feed to final effluent in both lines (not total P since there was not further release of P anywhere in the process).

Table 30: Soluble P average reduction using glucose mix

	Average Reduction (%)	Std. Deviation (%)
Feed to F.E. (A)	**86.34**	**5.00**
Feed to F.E. (B)	**63.08**	**10.00**
Feed to F.E. (AII)	**94.68**	**3.00**
Feed to F.E. (BII)	**71.45**	**11.00**

Line (A) was only the data from glucose-mix since milk was too short for statistics

It was thought that the viscous sludge encountered was either filamentous or zooglea bacteria, but researchers such as Gaval & Pernelle (2003) and Vaiopoulou *et al.,* (2007) mentioned in their research that the filamentous bacteria found in their anaerobic tanks were related to the recycling between the anoxic/aerobic and the anaerobic tank. Besides the absence of this recycle loop, there were other observations that did not concur with the literature on these bacteria proliferations (see Section 2.5.8.7)

- Since in our laboratory scale the only recycling was between the anoxic and the aerobic tanks then, the nitrate or the oxygen, could not be recycled into the anaerobic tank to encourage either filamentous or extracellular polymers (EPS) growth;
- The injection of influent was made directly into the MLSS tank, and the mix was very gentle with the paddles below the surface to avoid unwanted aeration;
- The viscous material growth only occurred in the anaerobic (3rd chamber) and did not develop in the anoxic or in the aerobic zones. EPS production has never been observed under strict anaerobic conditions (Miqueleto *et al.,* 2009);
- Normally filamentous bacteria growth occurs when the COD is low, but the MLSS growth in the anoxic and the aerobic tanks suggested adequate TOC (See TOC results in Annex I and II).

The descriptions of the MLSS withdrawn in this experiment are similar to those reported by the description of Zooglea made by Montoya *et al.* (2008), but the microscopic and superficial examination of the viscous MLSS suggested also filamentous bacteria. Filamentous strains, morphologically classified as Eikelboom type 021N bacteria was made by Kanagawa *et al.,* (2000) in nutrients removal plants which demonstrated the problem (See Figure 5, literature review section 2.5.8.7).

Research made by Kampfer *et al.,* (1995) tested 68 strains of filamentous bacteria concluded that all isolates grew better with d-fructose and d-glucose (monosaccharides) than, for example, using acetate as sole carbon (except Eikelboom type 021N). The degradation of complex organic substrates such as milk is a multistep process that involves several groups of degradation intermediates that might imply a greater diversity of microorganisms (in contrast to simpler substrates such as glucose) reducing the risk of filamentous bulking.

The other possibility was the viscous substance was green microalgae with rheological properties close to the EPS as it is function of biomass concentration (from 0.5 to 80 kg/m^3) (Woertz *et al.,* 2009; Wilemana *et al.,* 2012). There were other observations supported this hypothesis:

- The reactors were normally completely exposed to the light;
- High level of nutrients with calcium, magnesium, sulphur etc.;
- Little interference from VFA since algae are photosynthetic microorganisms;
- Presence of CO_2;
- The production of oxygen could explain the anoxic RedOx reduction noticed in the experiment (Figure 80)

However it was difficult to identify which the strain of microorganisms that grew in the laboratory rig, as this would have been a major undertaking. Moreover, the laboratory equipment and the time allowed were not sufficient to identify the microbiology. Given the novel process flow sheet further microbial analysis is recommended

In conclusion the presence of the viscous material was not detrimental in our experiments but problems would be expected from filamentous bulking with low F/M ratio in a multistage wastewater treatment (laboratory, pilot, full-scale plant) for nutrient removal (Vaiopoulou *et al.,* (2007), and Montoya *et al.,* (2008)). In our case this problem was overcome by the membrane, the viscous slime-like material mainly affects settling. Thus this could be recommended that, although not directly contributing to soluble P removal, membranes would improve the performance of EBPR plantsby removing the risks associated with settling.

While waiting for the new ICP and all samples were stabilised in acid, some test were made on other machines, among them. ICP with original process such as, for example, microwave ICP plasma using nitrogen as plasma (N_2-MIP-AES) (slightly colder plasma) rather than argon plasma used on ICP-OES. Results were not consistent providing differences with magnitude 10 times more. From the literature, it was mentioned that Ar-ICP-OES gives better results than N_2-MIP-AES (Ohata & Furuta, 1998).

6.5 Experiment Phase IV – using milk.

6.5.1 Introduction

This phase was carried out for 8 weeks, from 20/06/2012 to 13/08/2012, to assess two different amount of milk addition as exogenous carbon source (from the 20th July to the 27th July at 1% and from the 27th July to the 13th August at 0.5%). In the following figures, a discontinuous line has been used to separate the periods where CC1= 1% milk and CC2= 0.5% milk.

The reduction in the extra carbon source was primarily done to limit the production of MLSS noticed in the previous experiments, and to identify a sustainable effective standard food to microorganisms (F/M) ratio. Before starting the monitoring period experiment, the laboratory rig was run for 1 week to calibrate the analysis and allow the micro-organisms to acclimatize the new conditions.

The experiment was primarily out using a single rig line. The system was exactly the same as described above in previous section 6.4.1.1. To feed the laboratory continuous flow, fresh settled sewage from the local WWTW (Loughborough) was collected every Friday as previously to ensure a known strength throughout the week.

The Dionex (the apparatus used to measure the NO_3 concentration analysis in samples), was not either replaced or fixed. As the thesis main objective was to reduce the phosphorus, the nitrate was not fundamental but provided valuable information on its impact and RedOx support hypothesis during the discussion (NO_3-N for DPAOs). Moreover, the data would lead to an improvement in both final design and research future.

The chemical injection load remained at a flow rate of 200 ml/day of $CaCl_2$, 250 milli-molars (mM). The calcium was fed into both the anaerobic tank and the aerobic tank of the study rig. There were two wastage periods for MLSS reduction because of the high sludge concentration these are as described in Table 31 and Table 32.

Table 31: Anaerobic MLSS content

16/07/2012		Total Solids (g/L)	Ashes (g/L)	Volatiles (g/L)
Before removal	**AN (A)**	**13.39**	**2.29**	**11.10**
After removal	**AN (A)**	**5.68**	**1.29**	**4.39**
01/08/2012				
Before removal	**AN (A)**	**13.96**	**3.71**	**10.25**
After removal	**AN (A)**	**4.18**	**1.66**	**2.52**

The MLSS in the anoxic tank was also reduced on the 08/08/2012. The wastage process from the MLSS was as follows:

1. After emptying the anoxic tank, 10 litres of the original anoxic supernatant was returned into the tank;
2. 2.5 L of feed (the 08/08 settled sewage enriched with P) together with of 2.0 L of non-enriched settled sewage;
3. 0.5 L of the anoxic tank settled MLSS was returned to give a starting MLSS of minimum 5 g/Litre.

Throughout the experiment, there was recycle of MLSS from aerobic to the anoxic tanks, which balanced their MLSS concentrations. Therefore, it was necessary to waste sludge only from the anoxic tank to reduce the aerobic MLSS concentration. Table 32 shows the concentration before (2 days before 06/08/12) and after 24 hours after (09/08/12) sludge wasting.

Table 32: Anoxic and aerobic MLSS content

		Total Solids (g/L)	Ashes (g/L)	Volatiles (g/L)
06/08/2012	**AX (A)**	**5.32**	**2.33**	**2.99**
(Before removal)	**AE (A)**	**5.59**	**2.71**	**2.88**
09/08/2012	**AX (A)**	**3.03**	**1.54**	**1.49**
(After removal)	**AE (A)**	**2.67**	**1.55**	**1.12**

There were the following three sludge wasting:

1. Sludge wasting from the anaerobic tank:

- Sub-period I represents MLSS regrowth from the 15/07;
- Sub-period II represents MLSS regrowth from the 31/07.

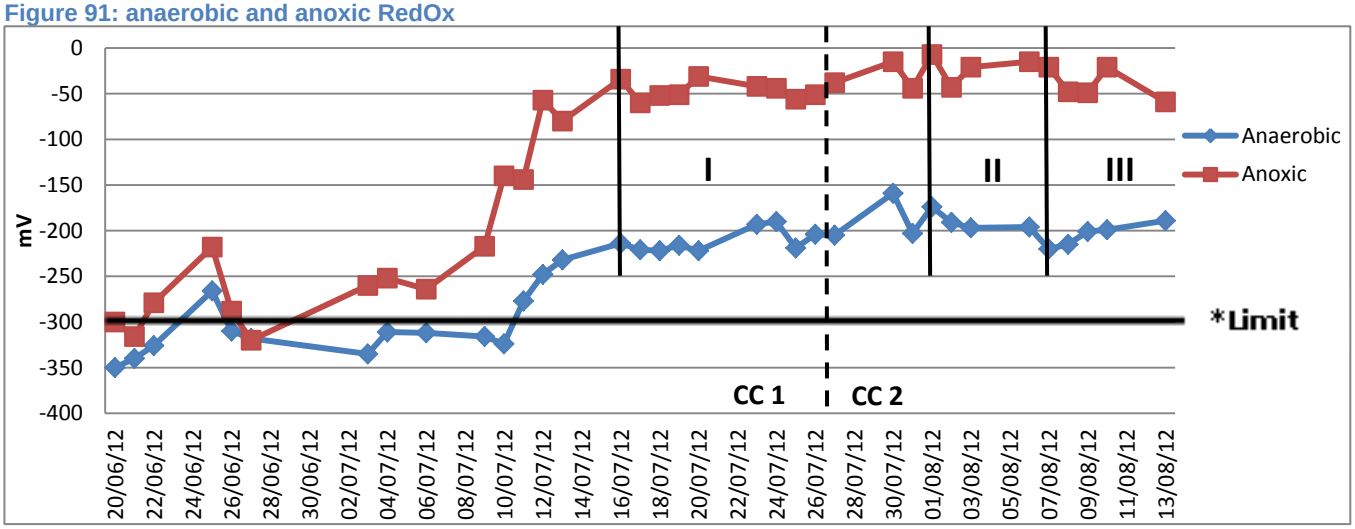
2. Sludge wasting from the anoxic (and aerobic) tank:

- Sub-period III represents the fourth (and last) MLSS regrowth from 07/08 until the end of the experiment.

6.5.2 RedOx.

The anaerobic and anoxic Redox curves are presented in Figure 91.

Figure 91: anaerobic and anoxic RedOx



***Limit:** below limit, the RedOx is considered as anaerobic condition**;** **CC1**: 1% milk concentration: from 20/06/12 to 26/07/12; **CC2**: 0.5% milk concentration from 27/07/12 to 13/08/12; **Sub-period I:** from 15/07/12 to 31/07/12; **Sub-period II:** from 01/08/12 to 13/08/12; **Sub-period III:** from 07/08/12 to 13/08/12.

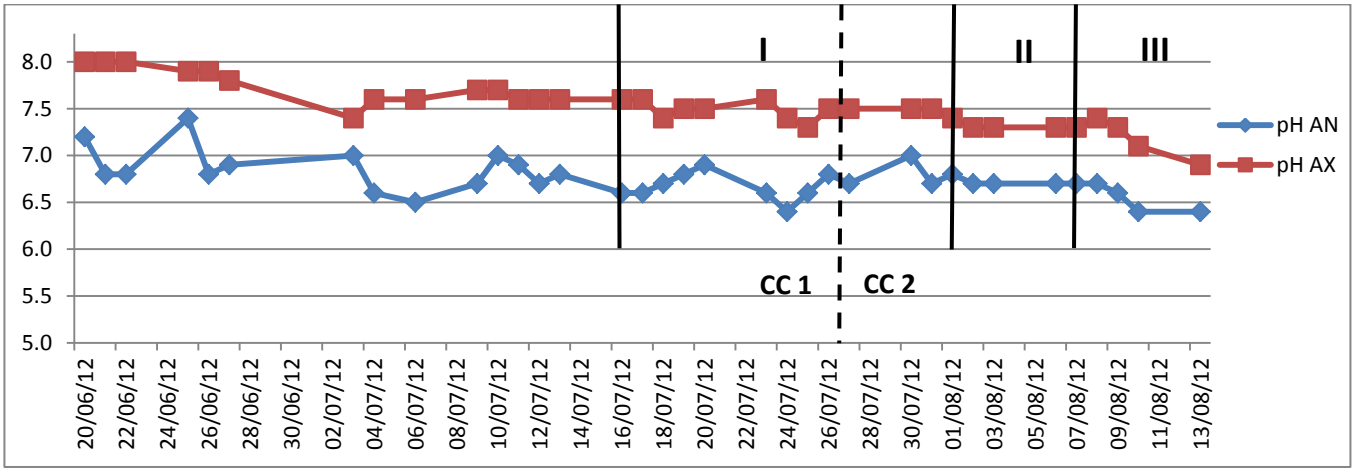
From the 20/06 to the 10/07 the anaerobic Redox was below the set limit of – 300 mV but then increased slowly to – 200 mV and remained consistently around this level. Compared to the previous experiments using milk, it is the first time that the anaerobic zone has failed to maintain anaerobic conditions. It was observed that the sludge was less 'dense' and lighter brown compared to what had grown in the other experiments.

When analysing the anoxic RedOx curve, in Figure 91, the initial anoxic curve was very low (close or below – 300mV), it was very low compared to what was wanted as it was anaerobic. However, from the 09th July the biological adjustment occurred so that the RedOx was closer to definition of anoxic conditions (Alberts *et al.*, 2008). The reduction of milk strength (27th July) did not affect the anaerobic nor the anoxic RedOx suggesting it was the MLSS concentration rather than the feed strength that controlled the RedOx.

6.5.3 pH from anaerobic and anoxic experiment.

.

Figure 92: Anaerobic and anoxic pH curves



CC1**: 1% milk concentration: from 20/06/12 to 26/07/12;** CC2**: 0.5% milk concentration from 27/07/12 to 13/08/12;** Sub-period I: **from 15/07/12 to 31/07/12;** Sub-period II: **from 01/08/12 to 13/08/12;** Sub-period III: **from 07/08/12 to 13/08/12.**

The anaerobic pH curve in Figure 92 has a decreasing trend and was mostly acidic 6.5 and 7.0. Compared to the anoxic the curve followed the same pattern but about one pH unit higher. This would be expected from the generation of VFA in the anaerobic zone. There are two important changes regarding the operation of the anaerobic tank in order to complete the discussion (see Figure 91):

1. First, the 16th July withdrawal of excess MLSS from the anaerobic tank, the total volume removed was equivalent to 3 litres (as 7.5 g/L). The liquid volume was replaced by settled sewage (not enriched with phosphate). This was not coincident with the increase in RedOx that took place and stabilised before.
2. The second wastage on the 1st August was different. The sludge was pale, but still brown. There was no effect on RedOx and it was decided to intervene by mixing the sludge from the anaerobic tank with the anoxic MLSS. This method was chosen because in WWTW it is expected to have sludge recycling from the anoxic tank to the anaerobic tank.

   The wasting and mix procedure were as followed:

   1) Emptying the anaerobic tank (5.8 litres);
   2) Putting back the separated anaerobic supernatant with 1.5 L of settled anaerobic MLSS;
   3) Adding 1.3 L of anoxic MLSS into the anaerobic tank
   4) The anoxic as oppose to the anaerobic tank received 1.3 L of not enriched phosphorus settled sewage.

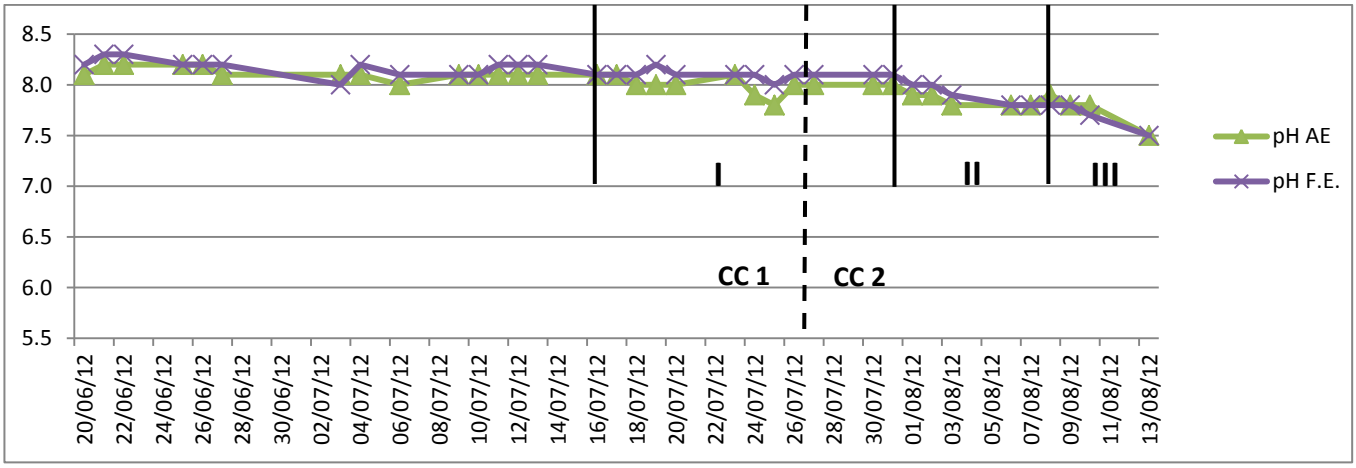
These changes did not modify the RedOx potential (Figure 91) for either anaerobic tank or anoxic tank, but arguably the MLSS changes did it affect moderately the anaerobic or anoxic pH level as it was described by the 3 following stable trends:

1. Mostly higher than pH 7.5 before 16th July;
2. Around pH 7.5 after the 16thJuly;
3. Below pH 7.5 after the 1st August.

Compared to the pH trend in Phase II and Phase III, the anaerobic tank was always more acidic than the anoxic as found in Phase IV. In Phase IV there is a trend to anoxic pH decrease toward pH 7.0, this might be as the consequence of the reduced milk concentration.

6.5.4 pH in aerobic tank and final effluent..

Figure 93: pH aerobic and F.E.



CC1: 1% milk concentration: from 20/06/12 to 26/07/12; **CC2**: 0.5% milk concentration from 27/07/12 to 13/08/12; **Sub-period I:** from 15/07/12 to 31/07/12; **Sub-period II:** from 01/08/12 to 13/08/12; **Sub-period III:** from 07/08/12 to 13/08/12.

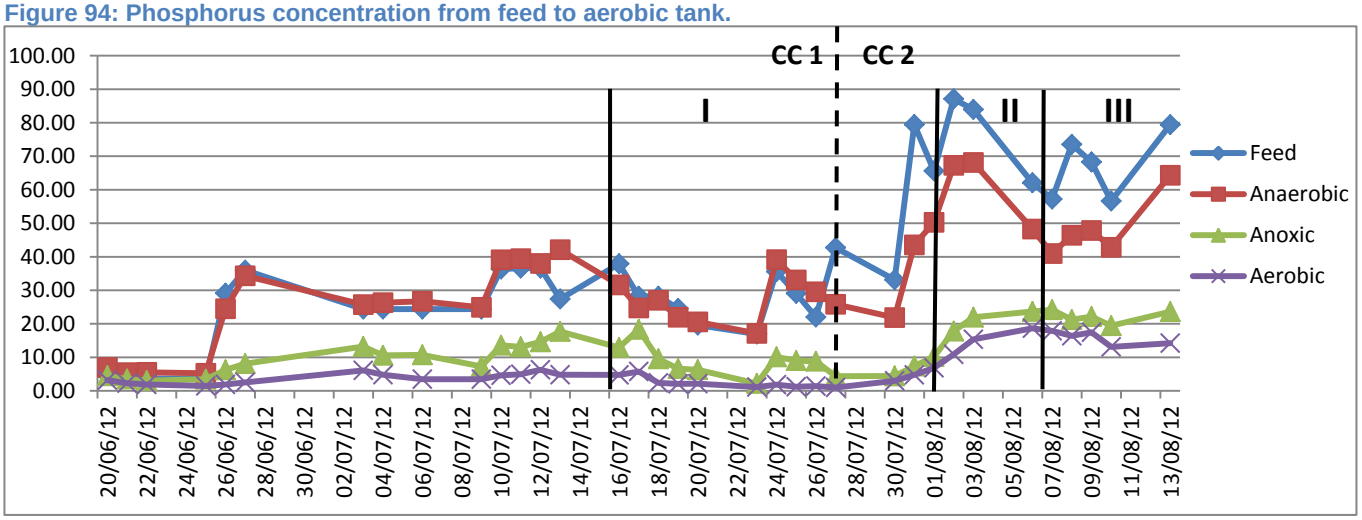
Figure 93 shows the pH from the aerobic and FE tanks that were alkaline. The pH curves, for the aerobic and final effluent, showed there was a reduction after the MLSS wastage made on the 31st July (Sub-period II). These trends in Phase IV repeated the same behaviour as in previous Phase II and III. The final effluent was mostly above pH 8.0 (before sub-period II), and as it was already noted in previous experiments, this pH level provided good conditions for precipitation and next step for nucleation process.

6.5.5 Phosphorus

6.5.5.1 Phosphorus content before final effluent.

The soluble P concentration in all the tanks except the final effluent is represented in Figure 94. The phosphorus was measured every day (except the W.E.) and therefore at every stage.

Figure 94: Phosphorus concentration from feed to aerobic tank.



CC1: 1% milk concentration: from 20/06/12 to 26/07/12; **CC2:** 0.5% milk concentration from 27/07/12 to 13/08/12; **Sub-period I:** from 15/07/12 to 31/07/12; **Sub-period II:** from 01/08/12 to 13/08/12; **Sub-period III:** from 07/08/12 to 13/08/12.

Extra P was not added to the feed until the 26th June and during the 1% milk concentration test period, it was decided to keep the phosphorus concentration in the range of 25 mg/L and 40 mg/L (± 5 mg/L) CC 1 Figure 94. Two main reasons drove this decision.

Firstly it was not easy to keep a steady concentration throughout the time with non-synthetic sewage. Every two weeks, the bottom of the feed tank was cleaned to remove settled solids that came from the real sewage. This avoided resolubilisation of P from the sediment and made the P concentration more predictable. It also avoided solids entering the peristaltic pumps which reduced the calibration necessary for the feed rate. A minimum of 25 litres was always needed to provide a constant head in the influent tank. As it was noticed that from day to day the soluble concentration of phosphorus had a tendency to decrease slowly in the stock feed influent, and an adjustment stock solution was prepared (20 litres) every two days to recalibrate the existing stock present in the tank. Final soluble P was the result of the mix solutions of the enriched prepared settled sewage and the existing soluble P in the already present sewage in the tank. Precipitation or take up by the microbial growth would explain the slow reduction in P concentration.

Secondly for the first experiment the desire was to focus on more realistic P concentrations that might be common in wastewater that treatment works might have to deal with. This aim lasted only one week without any addition of phosphorus. This first week provided a very low concentration (i.e., less than 5 mg/L in feed, in the week tested and the samples from the following weeks were almost similar). Thus it was concluded that the native P concentration for the study was firstly too low to provide useful data for the research. It had already been established that a W.W.T.W. could easily achieve the EU standard of soluble P at this concentration level. This was cnfirmed both in the literature (Akin & Urgulu, 2003; Neethling *et al.*, 2005) and in our earlier experiments (sections 6.2.2.7 and 6.3.9.2).

Moreover, variations in strengths are likely to be more common than a steady state due to storm water or population activity for example. Thus the best option was to artificially increase the P concentration in the feed to exaggerate the responses to milk as extra carbon source.

Until Monday 30th July, the concentration of soluble P was kept in the range (25 mg/L – 40 mg/L), the week-end (27th – 30th July was used as a buffer period to provide the system an adaption allowance to the reduced amount of extra-carbon source (from 1.0% to 0.5%). After the collection of data on Monday 30th, the feed strength P was increased to the limit between 55 mg/L and 90 mg/L, and thus being coincident with the reduced extra carbon. The idea behind that strategy was to add data to determine the quantitative link between P and the milk and establish a possible C:P ratio.

The anaerobic curve was equal to or slightly higher than the feed curve when the milk concentration was at 1% (Figure 94 CC1). As it was found in the earlier Phase II (1% milk) (Section 6.3.9.1 experiments) if the concentration of soluble phosphorus was above 50 mg/L, then the anaerobic curve started to differentiate itself from the feed curve. Below 50 mg/L the soluble P remained almost all the time equal to the P concentration as found in the feed (see in Figure 94). This response to a high level of soluble P might come from a change in the speciation of P that has increased its solubility. This has been despite the extra Ca^{2+} available to form calcium phosphate complexes. At a lower milk concentration a gap between the P curves was created.

It can be concluded that the reduction in P concentration (from feed to anaerobic Figure 94) came partly from the reduction in P from the lower addition of milk and also fixed retention of phosphorus inside the bacterial flocs. The P reduction from feed to anaerobic curve cannot entirely be explained by the reduction in the P in the milk component as the milk does not add more than 10 mg/L (as described in Figure 94 at 0.5%).

Nonetheless, reducing the milk concentration clearly reduces the soluble P in the anaerobic tank, suggesting greater precipitation since micro-organism growth and activity will be lower. This provides more opportunities to the P to be inorganically bond to calcium ions. The result in Figure 94 could concur with the idea that 1% milk could be too strong in the anaerobic tank, and thus creating sludge milk by-products that increase the MLSS excess interfering with potential chelating agents.

Unfortunately, the detail analysis of the different P species (Section 2.6.7.2 Table 5 and 6) was beyond the resources available, but is necessary in further work to trace the exact mechanisms. Nonetheless, from tests earlier done in this study, namely 1% milk concentration in Phase II and partly in Phase III, higher soluble P concentration created a difference between anaerobic and feed curves as microbial P, organic P and inorganic P, phosphorus and calcium reached a super-saturation state, and precipitation of calcium phosphate was started.

It is to point out that the reduction of MLSS done in sub-period I and sub-period II (anaerobic MLSS reduction in Figure 93) did not affect the performance process in any way. On the other hand, the reduction of milk did mechanically reduce the soluble P content immediately when the analysis is focused on the 27th – 30th July (Friday to Monday data). However this apparent change could also result from the high MLSS concentration where microorganisms had to compete for soluble substrates, and as the environment changed during this period of time both the RedOx (Figure 92) and the pH (Figure 93) were naturally adjusted. Particularly for the RedOx, the second reduction of MLSS (sub-period II) improved the electronegativity, suggesting an environment more adapted for a higher activity from the microorganisms.

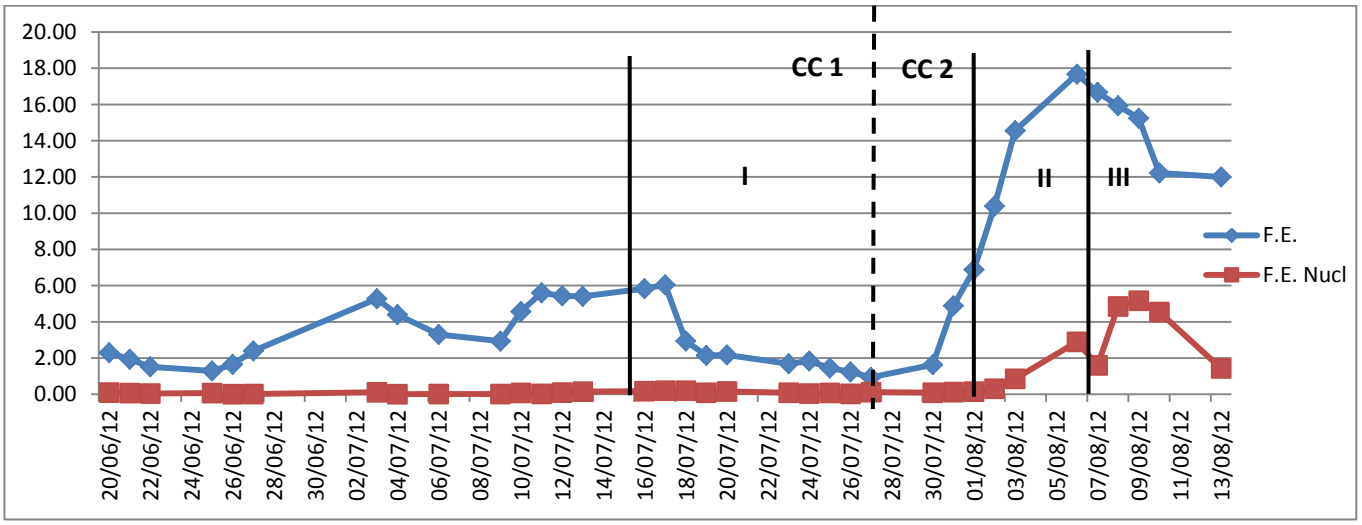
The anoxic curve (Figure 94) was also sensitive to the soluble P discharged from the anaerobic tank but the curve was dampened compare to the anaerobic and feed curves. The reduction of the anoxic-aerobic MLSS (sub-period III) did not affect the anoxic soluble P removal. However, the RedOx potential deteriorated slightly by, in average, 50 mV after milk reduction (Figure 92). This could suggest that the reduced extra carbon source was not enough, and then slowed the biological process of the microorganisms.

The aerobic curve was always lower than the anoxic curve and not very sensitive to the P discharged from the anaerobic tank effluent as it remained below 10 mg/L as long as the initial influent was below 50 mg/L, suggesting the equilibrium P uptake conditions. At higher soluble P influent feed concentration (between 55 mg/L and 90 mg/L), the aerobic curve went above 10 mg/L but lower than 20 mg/L.

The aerobic data figures should be related to the soluble P within the feed to propose any conclusions but arguably, it is difficult to state that the reduced milk and MLSS had adverse consequences on the further reduction of soluble P. The difference between the aerobic and the anaerobic P concentration are also important in sub-period II.

6.5.5.2 Phosphorus content in final effluent

Figure 95: Phosphorus concentration in final effluent and after nucleation

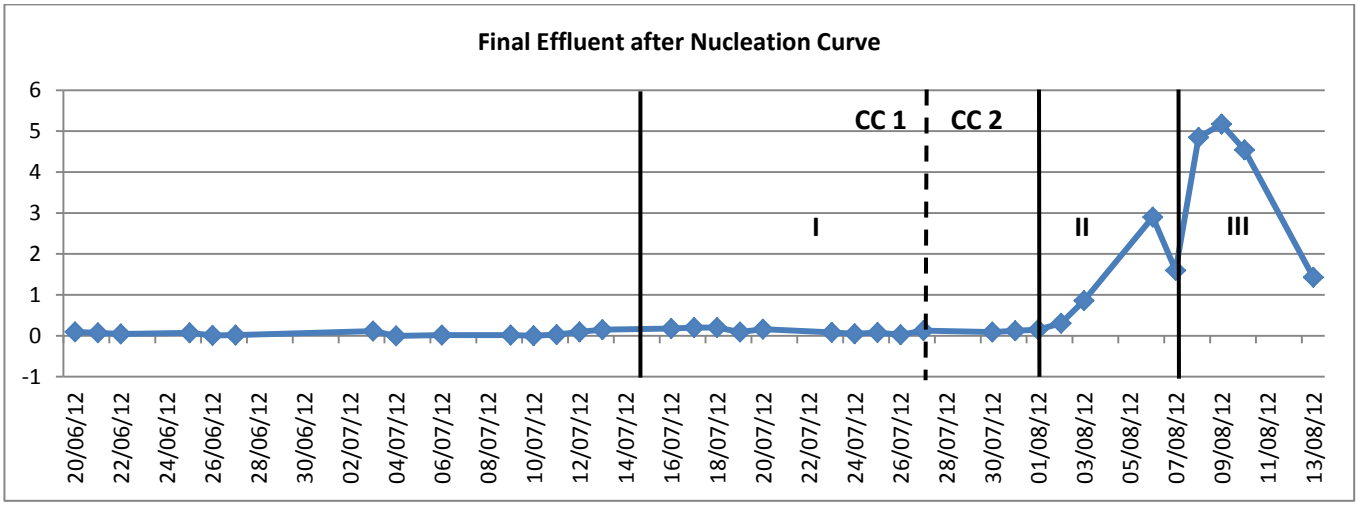


CC1: 1% milk concentration: from 20/06/12 to 26/07/12; **CC2:** 0.5% milk concentration from 27/07/12 to 13/08/12; **Sub-period I:** from 15/07/12 to 31/07/12; **Sub-period II:** from 01/08/12 to 13/08/12; **Sub-period III:** from 07/08/12 to 13/08/12.

Analysing the soluble P value of final effluent (F.E.) in Figure 95, the F.E. curve is almost identical to the aerobic curve, therefore this confirms, as seen before in the study, that the submerged membrane (0.4 μm pore size) was not reducing the soluble phosphorus concentration of the permeate. The F.E. curve reached its peak at 6.05 mg/L when the feed influent P concentration was kept below 50 mg/L, and the F.E. reached the peak of 17.7 mg/L when the feed was 50 – 100 mg/L P. Similarly to the aerobic curve analysis, the reduction of milk and MLSS did not perturb the F.E. P concentration already established before these changes. This is as in previous experiments where the final effluent P concentration follows the curve pattern set by the aerobic P.

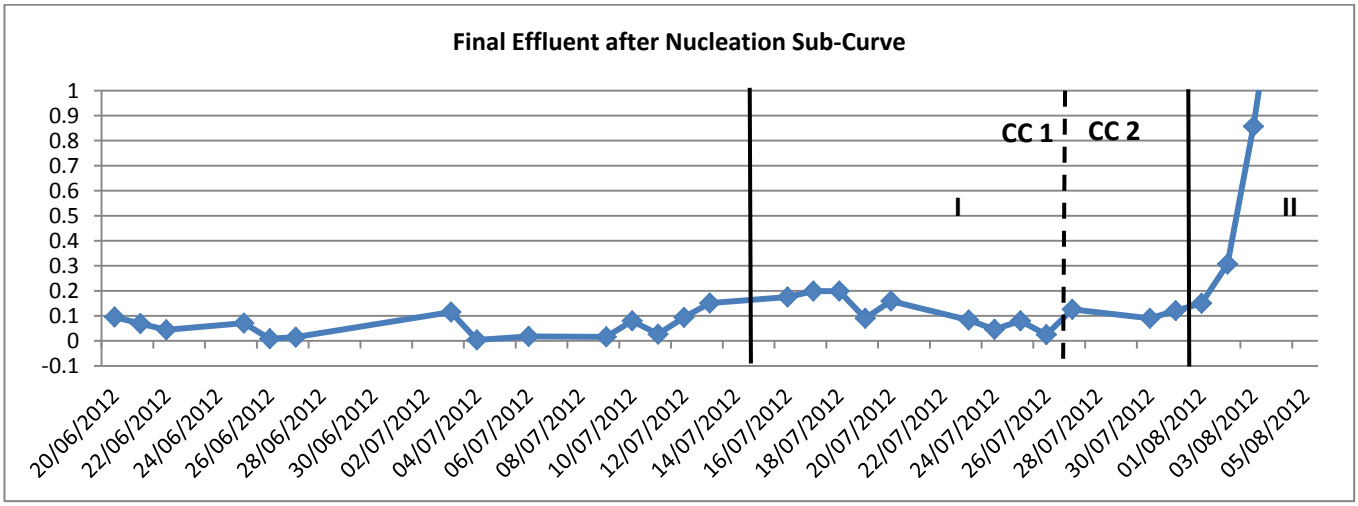
The curve of the final effluent after nucleation (F.E. Nucl.) treatment is shown in Figure 96 and Figure 97 and to be beneficial as the F.E. data prior to nucleation was 10 mg more far greater than 85 % of the time compared to the pre-nucleation data. Figure 97 is refined data which transfers all data where values did not meet the maximum of 1 mg/L of soluble.

Figure 96: Phosphorus concentration in final effluent after nucleation



CC1: 1% milk concentration: from 20/06/12 to 26/07/12; **CC2:** 0.5% milk concentration from 27/07/12 to 13/08/12; **Sub-period I:** from 15/07/12 to 31/07/12; **Sub-period II:** from 01/08/12 to 13/08/12; **Sub-period III:** from 07/08/12 to 13/08/12.

Figure 97: Sub-curve of phosphorus concentration in final effluent after nucleation (< 1mg/L)



CC1: 1% milk concentration: from 20/06/12 to 26/07/12; **CC2:** 0.5% milk concentration from 27/07/12 to 13/08/12; **Sub-period I:** from 15/07/12 to 31/07/12; **Sub-period II:** from 01/08/12 to 13/08/12; **Sub-period III:** from 07/08/12 to 13/08/12.

Table 33 shows the general phosphorus removal performance from the laboratory experiments Phase IV. The feed and the anaerobic to final effluent after nucleation are reported in the table.

Table 33: Average reduction of soluble P

	Milk Dose	Average Reduction (%)	Std. Deviation (%)
Feed to F.E. (A)	(1.0%)	82.76	13.10
Feed to F.E. (AII)	(1.0%)	99.45	0.60
Anaerobic to F.E. (AII)	(1.0%)	99.54	0.42
Feed to F.E. (A)	(0.5%)	84.06	8.62
Feed to F.E. (AII)	(0.5%)	97.01	2.95
Anaerobic to F.E. (AII)	(0.5%)	96.07	4.20

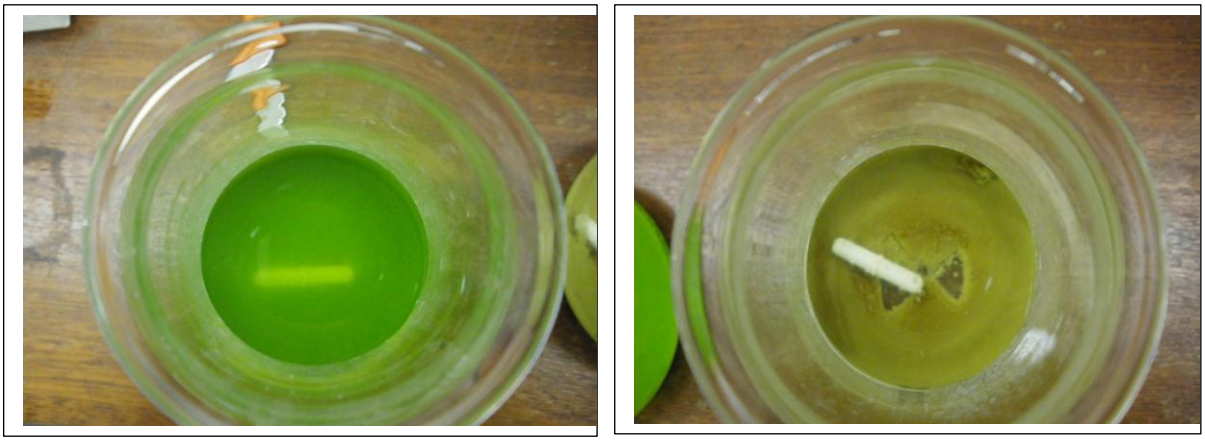
When compared to the soluble P concentration in final effluent, the curve after nucleation treatment was constantly insensitive when F.E. discharge was up to a maximum of 10 mg/L. This way the boundary of 10 mg/L in the final effluent is to be understood from the perspective of our tests are liked to the calcium and milk added.

After all processes before nucleation of keeping a large amount of phosphorus in the sludge (by absorption, adsorption), the remaining soluble P in excess still possess properties enabling it to be adsorbed by the calcium phosphate and then nucleate.

The boundary has been fixed at 10 mg/L in F.E. as after this amount, a slow increase started to be noticed. However, the rise was decoupled for two reasons, firstly because the augmentation/reduction did not start directly but after 1 or 2 days, and secondly the degree of increment/decrement was much lower than in the F.E. It is interesting to see that the constant value of 12 mg/L (11th and 13th August) led to a general reduction of the soluble P after nucleation (respectively 4.5 mg/L and 1.5 mg/L), most probably because it was close to the solid limit of the system.

For the nucleation process 500 ml of F.E. was collected to be poured in the nucleation tank reusing the calcium phosphate for several weeks. Only the presence of excess algae led to a cleaning of the tank and a renewing of calcium phosphate. The Figure 98 below provides an idea of what happened and when it was considered to be changed.

Figure 98: Nucleation tank containing algae



6.5.6 Conclusion for this final Phase IV

There are two ways to interpret the efficiency of our laboratory system.

- First the conclusion that soluble phosphorus in the F.E. did not achieve the standard for discharge. In this regard, the modified standard BPR with the addition of membranes, milk and nucleation was unable to consistently achieve a maximum of 1 mg/L irrespective of milk concentration and calcium.
- The second conclusion could be that given the extra P added both in the recipe and from the milk that the system could meet the standard by more controlled additions of other organic effluents and simple additional nucleation rather than coagulation.

Interestingly, the system was very stable even after reduction of MLSSs (anaerobic and anoxic-aerobic) the efficiency remained constant suggesting precise control when using the membrane.

A concentration of milk lower than 0.5% as external carbon source could be seriously considered because there was no evidence of lowering microbial activity. The beneficial elements seemed to be present in sufficient quantity. The second significant advantage of better control of MLSS would be the reduction in general calcium ion [Ca^{2+}] demand for action on soluble P.

7.0 Discussion

In this chapter, the results and discussion from the previous chapters are brought together in relation to the relevant objectives.

Over a two year period, a continuous flow laboratory scale activated sludge plant was used to test and compare the use of milk and a glucose mix, with and without calcium chloride additions, in parallel trains. Samples were analysed every working day for soluble P, nitrate, TOC, turbidity, pH, RedOx, MLSS and VSS.

7.1 Objective 1: Review of information of competitive processes for phosphorus removal and comparison with calcium chloride supplemented EBPR;

The most common chemicals identified for enhanced P removal were alum, ferric chloride and ferric sulphate. These three coagulants were very efficient for the total P and turbidity reduction when the pH was controlled (adjustment) and good mixing was achieved (Matilainen *et al.,* 2010). Alum degrades at lower temperatures and is rare naturally whereas toxicity and temperature are less important for ferric treatments (Matilainen *et al.,* 2010). Iron and Aluminium corrosivity was also important because both are more efficient at pH 4.5-6.0.and this pH is detrimental for microbiology and final discharge (Duan and Gregory, 2003; Matilainen *et al.,* 2010). Good total P and turbidity reduction has been shown to need between 2- 3 times molar doses of the ion (Tchobanoglous *et al.,* 2003).

During our tests calcium chloride, was successful for complex formation, not coagulation and used at lower concentration. It did not require rapid mixing or pH adjustment to be efficient for phosphorus removal. Experiments were frost protected but not at controlled temperature, cold and hot weather provided similar results suggesting calcium solubility was not an issue. However the extra chloride in wastewater treatment could increase chloride ion corrosivity where they accumulate at slow velocity or stagnant water (GE Power & Water, 2013). Metals and concrete are vulnerable and the additional hydrolysis promoted in EBPR produces H^+ ions which increases acidity (Elsener and Angst, 2007). During our experiments metallic parts did not exhibit corrosion and this could be due to the relatively rapid HRT (10 hours) and the dilution of chloride. This may still be a problem at full scale however given the variability in flow rates.

7.2 Objective 2: Assessment of Kubota® submerged flat sheet membrane bioreactor for P removal.

It was originally intended to complement this research with full scale data on P removal using membrane plant at Buxton WWTP but commissioning was delayed and. P data unavailable. This would still be useful however to confirm our result that the membranes did not improve overall P removal.

Kubota® recommendations for cleaning are to backfill the membrane with cleaner without lowering the tank level or removing the biomass. 0.5% sodium hypochlorite (NaOCl) is suggested every 6 months for organic fouling and yearly cleaning for inorganic fouling with 1% oxalic acid for Fe, or Al deposits or 0.5–3% HCl for $CaCO_3$ scaling. It is recommended that the solution remains and soaks the membranes for about an hour and then normal operation is resumed, without back-pulsing (Trivedi, 2004; Churchouse, 2005). This procedure was followed in the previous MSc.project work on the full scale plant which had noted problems from the iron used for coagulation precipitating on the membranes.

Kim & Yoon (2010) suggested that the order of chemical cleaning could be optimized to suit the conditions. Generally the acid solutions were used first to remove inorganic scaling stabilising the biofilm. However, our laboratory experiments show that NaOCl prior to the HCl could provide better removal of foulant, supporting Kim & Yoon second suggestion that cleaning should be optimized for each location. In our case the most probable foulant was thought to be a calcium-organic complex therefore, the bleach (NaOCl) loosened the organic biofilm allowing the acid to penetrate and react with the calcium scaling more efficiently.
Cleaning only with the HCl and bleach combination however never gave satisfactory results because after a further two or maximum three days the driving force needed from the peristaltic pump increased again, leading to air bubble formation in hoses. Cleaning was again necessary although it is important to note that calcium, was being added as part of the experiments. To restore the flux the membrane was removed for cleaning, this would represent an additional cost at full scale. It is recommended that further independent studies are needed to compare the full life costs of submerged with cross-flow membranes.

7.3 Objective 3: Link the soluble P removal with raw wastewater characteristics in comparison with the earlier reviewed work;

It was concluded that it was impossible to recreate a suitable synthetic wastewater to allow the normal complex and dynamic EBPR population to flourish. Adding real sewage also-improved the transferability into wastewater treatment at full scale. Moreover, domestic wastewater in western countries (see section 2.31) is similar, and thus, the results can directly be evaluated and criticised based on the process only. Seasonal changes in raw water characteristics were also integrated by collecting weekly samples from the local WWTW.

Test trials using sequencing batch reactors have been reported in the thesis but were not reproducible as seen Section 5.2. The main drawback for the SBR process was the constantly changing time dynamics. In the batch reactor sample collection was also a significant impact creating instability in operating conditions although it is accepted the reactor could have been made larger.

It was therefore concluded that the research would use a continuous flow laboratory rig which was simpler and more flexible to operate. These reactors proved successful. A major advantage of this form of operation was that it eliminated the transient conditions which were thought to be upsetting for the delicate PAO. The continuous flow configuration also allowed the use of on-line sensors although it is accepted that scaled up pilot plant would be even better and provide more data for statistical interpretation. Another advantage of the continuous reactors was the buffered responsiveness from the reactor size and stability from a constant flow rate. The experiments demonstrated that a response to change occurred to the results trends within 24 hours.

7.4 Objective 4: Perform laboratory scale bioreactor experiments to investigate and measure the effect of extra carbon sources on P uptake and MLSS growth rates.

7.4.1 Impact of exogenous carbon on RedOx and nutrients

7.4.1.1. Glucose, dextrin and soluble starch (glucose mix)

The literature review showed that simple carbohydrates were easily hydrolysed into glucose but the general consensus was that glucose was detrimental or created instability during EBPR (Mino *et al*., 1998). A metabolic excess of glucose favoured glycogen as main organic storage polymer and that the GAOs can accumulate and metabolize glycogen under anaerobic conditions faster than PAOs. The GAO consume sugars without involving PO_4^{3-}P in their metabolism (Randall *et al.,* 1997).

The dextrin and soluble starch in combination with glucose (i.e., the glucose mix), used in this research, was in contrast to the Mino and Randall results beneficial to enhancing EBPR, corroborating a more recent conclusion made by Gebremariam *et al*. (2012).

This more complex carbohydrate mix, as an external source, could have reduced the GAO advantage and these results suggest that complex carbohydrates could be a potentially competitive external carbon source.

<u>In the anaerobic reactor</u>

The laboratory rig from Phase II onwards, did not have the standard EBPR recycle sludge loop to the anaerobic tank found in the classic UCT process. Therefore there was no release from Poly-P into the supernatant by the PAO. The anaerobic tank was instead just enriched with readily assimilable carbon. This was also proposed by Randall *et al.* (1997), in particular, and others who recommended a simpler pre-fermentation and hydrolysis stage could suffice with sufficient soluble substrate to allow the EBPR to compete.

The literature model is that PAOs need to be a major part of the population if P removal is to be successful and this requires the take up small molecular weight acid substrates in order to produce poly-P. This generates acidity (Gebremariam *et al.,* 2012);

Previous literature has as a consequence of this pre-fermentation, suggested the pH becomes more acid (pH 5.0 – 6.5). For example Gebremariam *et al.,* (2012) and others such as Kargi & Uygur (2005) concluded that glucose as major carbon source was detrimental to pH level.

The external pH reduction is explained by the VFA metabolites of glucose. Bond *et al.* (1999) observed that once intracellular acidification occurred then there was inhibition of anaerobic phosphate release. There were several differences in our research, the most important was that anaerobic P release did not occur because there was no recycling sludge loop. Recycling alternates the sludge between anaerobic and aerobic conditions which could reduce stability. Our reactors also ran for a longer period of time than most in the literature (usually more than three growth cycles per phase) and the pH stabilised at 6.0 and remained stable as long as the MLSS was the same, despite higher concentrations of the glucose mix

In conclusion if the poly-P metabolism is avoided and GAO, OHO fermentative bacteria are promoted, this forces none PAO metabolic pathways and avoids pH reduction despite increased concentrations of glucose mix (Figure 47 and 60).

Anaerobic RedOx was not as easily achieved as expected in our experiments although it was lower in Phase III than in Phase I. It was concluded that acclimatisation time was the most important influence on redox (i.e., Phase III was operated over a longer period of time than Phase I) and although the anaerobic curve was unstable, it was nonetheless consistently lower than earlier experiments [– 200 mV; – 350 mV].

The design, the chemical characteristics of the wastewater, the source of MLSS and inoculation concentration are reported to be important factors in the eventual stable culture formed. To start the experiments, the MLSS along with the wastewater came from the local wastewater treatment works which had recently been reconfigured for EBPR. There was an equal distribution of this inoculum between the zones in the reactor.

Since there was no recycle loop involving the anaerobic reactor (biomass was retained by baffles) the most important selection parameters were the substrate concentration and the RedOx environment (Filipe *et al.,* 2001a,d). An example result of this was the evolution from an initial acidic, anaerobic pH curve during Phase I to neutrality which demonstrated a shift in microorganisms and adaptation over time:

In the anoxic and aerobic reactors

Achieving denitrification to ensure anaerobic conditions following aeration (constant O_2 concentration) was a common problem noted by the literature. In the case of membrane process assisted denitrification which was a function of the evolution of the MLSS and oxygen demand.

The influent after rain would still provide more aeration for an identical nitrifier population and worse nitrate removal than expected but this was not obvious on P removal because of the higher endogenous respiration from the high concentration of MLSS.
Residual substrates from the anaerobic tank were available in both the anoxic and probably aerobic tanks since the MLSS increased steadily in both zones. There was a conventional recycle loop to exchange MLSSs between the aerobic to the anoxic tank. At low substrate concentration the anoxic RedOx was variable (Phase I Period 1 and Phase III), but more stable (Phase I Period 2) as would be expected from the higher glucose mix concentration. In general the anoxic RedOx curves were close to or below zero and thus deprived from oxygen. The anoxic and aerobic pHs were both always above 7.0.

The nitrate concentrations were higher at lower glucose mix concentration [20 mg/L – 40 mg/L] corroborating previous work that the substrates provided were insufficient to denitrify and competition for AOM was a problem. At the higher carbon concentrations N removal was almost complete [0 mg/L – 20 mg/L], suggesting a least TOC:N ratio of 60 was needed in the anaerobic zone.

7.4.1.2 Effect of milk

General texts for biological treatment have suggested the ratio of COD:N:P in the wastewater to be treated should be approximately 100:20:5, for aerobic treatment, and 250:25:5 for anaerobic treatment (Henze *et al.,* 1997; Tchobanoglous *et al.,* 2003). For nutrient removal however the figures have been modified. Mulkerrins *et al.* (2004) stated that the consensus for efficient BPR, was that the COD:P ratio should be above 50 in the anaerobic zone. These guides are useful operational information, but the academic literature has indicated that only VFA can be stored in the cell by the PAO (Wentzel *et al.,* 1991; Lie & Welander, 1997; Mino *et al.,* 1998, Wang *et al.,* 2004). Thus COD and BOD lack precision in determining the efficacy of exogenous carbon. HAc and HPr are the dominant VFAs in raw domestic wastewater (Mino *et al.,* 1998; Oehmen *et al.,* 2007), and many academic bench scale studies have used HAc or HPr as sole carbon to improve EBPR (Kuba *et al.,* 1994; Smolders *et al.,* 1995; Oehmen *et al.,* 2006a, 2007). However, using HAc and HPr as sole carbons has, as already been noted, reported to cause instability in EBPR (Filipe *et al.,* 2001a; Oehmen *et al.,* 2007).

Thus it was decided at the beginning of the research to investigate other potential waste co-substrates and milk was chosen. It was hypothesised to be rich in potential VFA after the anaerobic fermentation and likely to be cost effective. It was also suggested that milk had potential chelating agents because it contains complexes to bind calcium and phosphorus to exceed their normal saturation.

In the anaerobic reactor

The results from our experiments have confirmed that milk was an efficient source of assimilable substrates. This corroborated the literature which showed that the milk fermented using the normal hydrolysis steps (Lucas *et al.,* 2007). The more complex milk degraded slowly compared to the glucose and was shown to provide a steadier yield of VFAs. A particular example was during the Phase III, when milk was used for a limited time to increase the poor P removal performance of the control line previously fed with glucose. The improvement was rapid and lasted for several days even after stopping the milk addition. These conclusions were corroborated by Fernandez *et al*. (2011), who reported that milk and tomato wastewaters, were the most suitable, from a range of food processing effluents to improve EBPR long term.

One of the targets of using an external source of carbon was also to achieve anaerobic conditions more easily. Using different strengths of milk in this research generated inconsistent results. There were some experiments where the RedOx was not reduced by increases in milk concentration, for example (Phase I Period 2 [2.5% concentration] higher redox compared to Phase IV [1% concentration then 0.5% concentration].

Links to temperature or biomass concentration and dissolved oxygen could not be found. The temperature in Phase III was during cold weather but the redox achieved was below –300mV, compared to Phase IV with hot weather and a worst electronegativity. There was also initially a greater MLSS concentration during these Phase IV experiments but the redox potential was – 200 mV. MLSS was then wasted to increase respiration rate but the redox was well buffered and did not respond.

Some possible reasons can be suggested which need further research:

1. These higher than expected redox were earlier in the experiments and later in potentially better acclimatized cultures the anaerobic redox was routinely between [–200mV and – 350mV].
2. The use of RedOx probes for control is not widely reported and their performance in this role needs further work.
3. The range [– 250mV; – 200 mV] could be an equilibrium RedOx linked to specific metabolic pathways using specific external substrates.

4. The pH was mostly within [6.5 – 7.0] and this lower pH was from both milk and glucose. The acidity could have limited the ion exchange and reduced microbial growth rate stabilising redox.

Despite these low anaerobic RedOx potentials, the data did confirm that the RedOx was low enough to allow the milk to ferment (redox was below 0 mV) and the pH decreased.

Classical BPR first uses anaerobic redox to release P and then aerobic conditions to promote excess uptake. It has been suggested that the lower the redox, the more $PO_4^{3-}P$ is released (Mulkerrins *et al.,* 2004). From our work we can conclude that, achieving anaerobic redox potential is not always necessary and P uptake can be linked to the availability of VFA.

The slow fermentation rate of milk also meant that there was little difference in the results between 1% and 0.5% milk concentration as both could achieve a sufficient amount of VFA. This should enable the amount of extra carbon to be limited to reduce excess sludge production and control pH and cost (i.e., milk supply, sludge management and aeration).

In the anoxic and aerobic reactors

There was recycle from the aerobic to the anoxic tank for denitrification which exchanged their MLSSs and bacterial pool. Unlike the anaerobic zone, the anoxic reactor maintained the ideal text book redox potential.

Identification of bacterial populations are now possible using genomics (e.g., fluorescence in situ hybridization (FISH)) which should make it possible to differentiate between bacteria and monitor changes in the ratio of PAOs, DPAOs, OHOs, and other diazotrophs. This would be recommended in further work. In this thesis however the coincident reduction of the key nutrients NO_3-N and $PO_4^{3-}P$ in both the anoxic and aerobic tanks were apparent.

Thus for the anoxic reactor it could be concluded that DPAOs were present in the bacteria population. Moreover, as the DPAOs and OHOs were capable of reducing the level of N and P, this was taken as evidence that the amount of VFA or other easily assimilable carbon (provided from the anaerobic reactor) is the main parameter controlling biological reduction of nutrients.

The pH optimum for denitrification was reported to be between pH 7.0 and 8.0 (Wang *et al.,* 1995; Mulkerrins *et al.,* 2004). Below pH 7.0, there is a significant decrease in rate of denitrification (Randall et al., 1992, Glass and Silverstein, 1998). The explanation is partly due to increasing accumulation of nitrous oxide (N_2O) during heterotrophic denitrification (Pan *et al.,* 2012).

A neutral pH was achieved in the anoxic and aerobic zones during all experiments and there was an increase in pH from the anaerobic to anoxic reactor of about 1.0 to 1.5 as the nitrates were stripped into N_2 gas (Zhu *et al.,* 2008) with partial P-uptake. Mekmene *et al.* (2009) noted that between pH 6.5 and 8.0 (ideally pH 7.0) when Ca/P ≥ 2, then phosphate concentration was the limiting factor in precipitation. The anaerobic pH was often acidic and may have contributed to preventing complete precipitation.

The denitrification rise in pH is well known from the stoichiometry, acidity is consumed for every mole of nitrate reduced to nitrogen gas. From the example using acetate as electron donor (Glass and Silverstein, 1998):

$$0.625CH_3COO^- + 1NO_3^- + 0.375\ H^+ \rightarrow 1.25HCO_3^- + 0.5N_2 + 0.5H_2O$$

In this example, a net 0.375 of acidity has been consumed to produce 1 mole of nitrogen gas.

During the experiments a further rise of 0.3 ± 0.1 was measured from the anoxic to the aerobic reactor and this has been linked to phosphate uptake and the CO_2 stripping (Gebremariam *et al.,* 2012). Therefore the ΔpH is indicating that the EBPR process is operating as more VFA are consumed. The aerobic curve was mostly at pH 7.8. Filipe *et al.,* (2001a) reported that PAOs started to lose their advantage at pH below 7.25, and Zhang *et al.,* (2007) found long-term EBPR operation was optimal at pH [7.6 – 8.0].

7.4.2 The phosphorus dynamic

In the anaerobic reactor

As it was mentioned earlier in the discussion the PAOs were not recycled into the anaerobic tank and for this reason, there was little or no release of poly-P. The use of milk as external carbon source clearly brought an additional soluble P in the MLSS since the anaerobic curves describing the soluble P content were always higher in the experiments using milk than those with glucose.

The increases in soluble P were differentiated by the experiments using milk alone without additional inorganic P (Phase I only the data and Phase III line (A), 1% milk concentration, where no calcium chloride was added to the anaerobic tank). No speciation analysis of calcium phosphate forms was performed during the thesis and this can be recommended for further work. However, the results suggest a transformation of the feed soluble P form into a less soluble form by a combination of nucleation and biological uptake making it easier to keep in the sludge.

The anaerobic curves using milk were only always lower than the influent curves when the level of soluble P was ≥ 40 mg/L. Whereas using glucose, a higher (80 mg/L in Phase III) or lower starting soluble P concentration (average 8 mg/L in Phase I Period 2),then anaerobic curves were not always consistently lower. Other results also provided evidence to support the conclusion that the response from the BPR process was important to overall P removal. For example in Phase III, the experiment using only $CaCl_2$ without exogenous carbon source, all the P curves (anaerobic to aerobic) were lower but inconsistent suggesting that, the first dose of $CaCl_2$ (250 μM), changed the saturation index. The further additions later in the process did not improve overall precipitation or the crystallinity. Unfortunately due to the decommissioning of the ICP and time constraints for access to X ray diffraction equipment, it was not possible to investigate the detail mechanisms of precipitation further. The $[Ca^{2+}]$ additions to the aerobic tank using calcium chloride was assumed to react with $[PO_4]^{3-}$ but other species present in wastewater, referred by Barat *et al.,* (2011), need to be considered in the future

For example Cao and Harris (2008) investigated the carbonate and magnesium effect on calcium phosphate precipitation and found that carbonate $[CO_3]^{2-}$ significantly reduced the precipitation rate by competing with PO_4^{3-} , the magnesium ion Mg^{2+} also severely inhibited precipitation rate and crystallinity and moreover there was a synergistic inhibitory effect when both elements where present. The literature and experimental observations therefore suggest that the milk can generate some additional precipitation but these processes are complex depending on the precise conditions. They are also incomplete without the assistance of BPR and soluble aqueous P remains.

In the anoxic and aerobic reactors

The conventional recycle loop was installed between the aerobic and anoxic tanks alternately exposing the same culture to both nitrifying and denitrifying biochemical pathways. Some PAOs (ηPAOs), also known as DPAOs, are capable of using both oxygen and nitrate, whilst others only of using oxygen (Hu *et al.,* 2002). (Section 2.5.8.7 in the literature review)

During this thesis, in all experiments where the influent phosphorus content was artificially increased (above 20 mg/L) the differences in soluble P between the anoxic reactor and aerobic reactor were negligible compared to those from anaerobic to aerobic effluents. The average difference between anoxic and aerobic reactors was also independent of the carbon source and its concentration, as well as the calcium chloride addition.

Thus it was concluded that P removal was biologically driven, corroborated by the data which confirmed previous work reviewed that without exogenous carbon, in the anaerobic stage low soluble P was not possible . Unlike most previous research however, P release in the anaerobic step was not necessary as long as AOM was present. During the experiments with wide variations in soluble P namely Phase IV (Figure 94) the final aerobic reactor buffered this impact to give a much smoother more desirable final effluent P. The slightly greater soluble P in the anoxic compared to the aerobic curves can be explained by the higher feed concentration from the anaerobic tank. A larger difference was expected between the anoxic and aerobic zones because the APAOs growth was reported as more important than DPAOs by Hu *et al.,* (2002). They predicted a further release of PO_4^{3-}P in the anoxic tank from APAOs generating PHA because they are not capable of using nitrate as electron acceptor. In our results this was not the case, the level of PO_4^{3-}P was the same in both tanks. Therefore there was no evidence of differences between APAO on DPAO activity despite the high nitrate concentrations. A better prediction of residual P was given by the concentration and complexity of the additional carbon rather than the level of redox condition (anaerobic, anoxic).

The aerobic (and similarly the anoxic) P curve was lower using glucose than milk because of the P content of the milk. Phase I Period 2 (Figure 63; Figure 64). Moreover, using the glucose mix it was possible to achieve the EU 1 mg/L (partially in period 1 and all the time in period 2).

Conclusions

1. Competition from the faster growing OHO was predicted to inhibit EBPR (Ekama & Wentzel, 1999; Hu *et al.,* 2002), and Filipe *et al.,* (2001 a,b); suggested this was linked to the intensity of redox, the lower the greater PO_4^{3-}P release was. The level of AOM substrates available in the anaerobic /anoxic reactors provides an opportunity for the APAOs to produce PHA as appose to PHB and thus release PO_4^{3-}P (Mulkerrins *et al.,* 2004). In our case however, we have concluded the VFA was in excess since there was P uptake and MLSS growth during all experiments.
2. It was also concluded that the aerobic P represented the soluble P species that could not be taken up by APAOs and the curve represented the residual PO_4^{3-}P which would need further treatment.

The Final Effluent

There was no improvement in the soluble P removal between the final effluent and the aerobic tank following the membrane. There may have been a slight improvement which was, more likely related to the retention time in the final effluent tank and time before analysis since this could be ± 24 hours.

The TOC in the F.E. was very low, the average was <10 mg/L except for phase I with the highest milk concentration 2.5% were the average was doubled (20 mg/L). The final effluent turbidity was between 0.2 – 0.5 NTU. Some days the NTU was higher than 1, but this was related to fouling of the sampling tubes (from microalgae mainly).

The membrane needed to be cleaned more often than recommended by the manufacturer. A constant flow rate was easy to maintain by increasing the peristaltic speed to compensate for fouling. Frequency of cleaning increased with the start of the calcium dosing in the aerobic tank. It was possible to feel small grains around the walls of the membrane.

Based on this observation the calcium chloride (Phase II) injection was moved into the anaerobic tank and this kept membrane efficiency for much longer. One of the membranes was thought faulty because, the clogging was twice as frequent as the three others despite the thorough cleaning (the cleaning was always outside the aerobic tank, see the details in the methodology).

Table 34 summarises the results from feed to final effluent for the continuous experiments. The results are expressed as % removed to avoid some of the bias from phosphorus that has been added. It was concluded that 0.5% milk could be as good as sugar additions shown by Phases III and IV.

Table 34: Average reduction of P from feed to final effluent in all Phases

	Average Reduction %	Std. Deviation	
Phase I Period 1, 1.0% ECF, $CaCl_2$: *NWPC; (A) = milk; (B) = GM*			
Feed to F.E. (A)	**84.71**	**5.70**	
Feed to F.E. (B)	**89.39**	**8.15**	
Phase I Period 2, 2.5% ECF, $CaCl_2$: *NWPC; (A) = milk; (B) = GM*			
Feed to F.E. (A)	**76.58**	**7.56**	
Feed to F.E. (B)	**95.81**	**1.24**	
Phase II, 1.0% milk ECP/API/*200 ml 250μM $CaCl_2$: (A) = in AE; (B) = in AN*			
Feed to F.E. (A)	**84.63**	**9.93**	
Feed to F.E. (B)	**83.93**	**14.12**	
Phase III, API, $CaCl_2$: *(A) = 1.0% GM; (B) = no ECP (only $CaCl_2$)*			
Feed to F.E. (A)	**86.34**	**5.00**	
Feed to F.E. (B)	**63.08**	**10.00**	
Phase IV period 1, $CaCl_2$: *(A) = 1.0% milk*			
Feed to F.E. (A)	**82.76**	**13.60**	
Phase IV period 1, $CaCl_2$: *(A) = 0.5% milk*			
Feed to F.E. (A)	**84.06**	**8.62**	

ECF: Extra Carbon Food; NWPC: Natural Wastewater P Concentration; API: Artificial P Increase; GM: Glucose Mix.

At the elevated P few of the F.E. data were below the 1mg/L EU standard, but since the glucose dosed line received less P overall it most consistently met the standard.

In Phase I Period 2 it was concluded that too much additional milk was added but the equivalent glucose equivalent worked better. Phase III calcium chloride only in Line B demonstrated the importance of EBPR compared to precipitation alone. Calcium chloride alone removed between 50% - 75% of soluble P from the wastewater.

At up to 110 mg/L, Phase III (see Figure 87 in), Line A with glucose soluble P reduction was quite stable around 50 mg/L, did not improve with time and never achieved the exceptional results earlier in Phase I.

Possibly the weather played a partial role since Phase 3 experiments were during colder temperature. The average percentage removal of soluble P using milk was constantly around 85%, irrespective of the $CaCl_2$ injection point or detail temperature. Restoring lower concentrations of milk (Phase IV using 0.5-1%), returned the performance.

The standard deviation in Phase lV was between 6% - 15%, and was related to the concentration of soluble P in the anaerobic tank not the external environment. The higher the P concentration was, the lower the variability.

Final Effluent after Nucleation

Table 35 summarises the P removal performance across all the stages including the final nucleation which was necessary to meet the international standard. The data shows that both the average soluble P and the standard deviation were reduced by the nucleation irrespective of the additional carbon source.

Table 35: Average reduction of P from feed to final effluent after nucleation in all Phases

	Average Reduction	Std. Deviation	
Phase I Period 1, 1.0% ECF, $CaCl_2$: *NWPC; (A) = milk; (B) = GM*			
Feed to F.E. (A II)	**96.58**	**0.71**	
Feed to F.E. (B II)	**97.43**	**0.39**	
Phase I Period 2, 2.5% ECF, $CaCl_2$: *NWPC; (A) = milk; (B) = GM*			
Feed to F.E. (A II)	**93.19**	**2.15**	
Feed to F.E. (B II)	**96.78**	**1.85**	
Phase II, 1.0% milk ECP/API/*200 ml 250μM $CaCl_2$: (A) = in AE; (B) = in AN*			
Feed to F.E. (A II)	**97.18**	**1.39**	
Feed to F.E. (B II)	**97.51**	**1.73**	
Phase III, API, $CaCl_2$: *(A) = 1.0% GM; (B) = no ECP (only $CaCl_2$)*			
Feed to F.E. (A II)	**94.68**	**3.00**	
Feed to F.E. (B II)	**71.45**	**11.00**	
Phase IV period 1, $CaCl_2$: *(A) = 1.0% milk*			
Feed to F.E. (A II)	**99.45**	**0.50**	
Phase IV period 1, $CaCl_2$: *(A) = 0.5% milk*			
Feed to F.E. (A II)	**97.01**	**2.95**	

ECF: Extra Carbon Food; NWPC: Natural Wastewater P Concentration; API: Artificial P Increase; GM: Glucose Mix.

- In Phase I, (extra carbon, and calcium addition) both the glucose mix and milk streams consistently achieved 0.5 mg/L, including the higher initial P concentration from the milk as supplementary carbon.
- In Phase II, (extra phosphate, and separate injection zone for $CaCl_2$) the soluble P in the final effluents almost achieved the same 0.5mg/L as Phase I final concentration was between 0.3 mg/L and 0.6 mg/L (the average was better in line (B)).

- In Phase III, comparison of glucose mix with the control $CaCl_2$ only, no results below 1 mg/L suggesting that the level of glucose food provided was too weak for the extra phosphate added or sufficient to achieve optimal biological performance as the results from Phase I Period 2 had better results when the glucose mix was increased.
- In Phase III the range of soluble P removal using 200 ml (250mM) of $CaCl_2$ was between 60% and 85% and the remaining soluble P average around 20 mg/L (maximum 36 mg/L). These were the worst P removal results recorded.
- In Phase IV using 1 % milk following nucleation were the best P removal results recorded, the initial soluble P in the feed was between 20 and 40 mg/L and effluent below 0.2 mg/L (mostly below 0.1 mg/L).
- In Phase IV using 0.5 % milk, with nucleation the P remained around 0.1 mg/L for 1 week as a result of carryover from the previous 1 % milk even when the feed P was above 85 mg/L. The F.E. P started to increase above 1 mg/L after 10 days following the milk reduction. Once steady state had been achieved then the average reduction of P was around 97 % and gave a final effluent of about 1 mg/L from an average feed of 50 mg/L (higher than in the first part of Phase IV).

8.0 Further work

8.1 Laboratory scale

Further investigations of the role of the Denitrifying Phosphorus Accumulating Organisms DPAOs) at bench scale should be carried out using a design modification to the rig used in these experiments :

- Creating a dedicated fermentation tank (hydrolysis tank) that releases mainly VFA separately into the anaerobic tank compared to tests using the standard recycle into anaerobic zone; the UCT process. This could promote P release not seen or tested in our research;
- Dedicated monitoring and control of the N mass balances in the anoxic and anaerobic stages. This could provide data on the little researched and slow growing DPAOs to determine their competitiveness of over the other hydrolysing organisms OHOs;
- The addition of another recycle loop from the aerobic tank to both the anaerobic as well as the-anoxic tank. This could provide an enhancement in biological phosphorus reduction since the results reported in this thesis suggest the PAOs were not involved in the anaerobic tank. The potential of Poly hydroxyl acetate (PHA) accumulation was probably not fully realised, and therefore reduced the overall potential biological phosphate uptake which could yet be enhanced;
- These increases in stages could be achieved as in our experiments with baffles within one tank.

8.2 Analysis

Identifying the biological phenotype, genomics and metabolomics of the PAOs and DPAOs;

Carrying more tests comparing other complex carbon sources since the glucose mix provided enhancement but not as good as milk.

A longer period of experiments during cold weather and without external addition of phosphorus is also needed.

Part of these experiments should include other food industry wastewaters and specific VFAs measurments (acetic, propionic,butyric, iso-butyric, valeric, iso-valeric, caproic, iso-caproic and heptanoic acids), and dissolved organic carbon release analysis to assess which are most suitable for EBPR wastewater treatment.

Further work to develop RedOx measurements for the control of P removal environments is needed.

8.3 Nucleation Experiments

More research to adapt the calcium chloride additions for domestic wastewater treatment with milk or other exogenous carbon source. Work to compare the saturation indices could be carried out with different using different wastewaters.

More detail analysis of the P speciation using for example X ray diffraction or electron diffraction would help understand the mechanisms and impact of changes in inorganic ion balances encountered, for example in hard waters and other sources of Ca.

More detail analysis of the calcium phosphate species formed when milk is used in:

1. The anaerobic tank:
2. The aerobic tank or final effluent tank;
3. The nucleation tank: to better understand how the soluble P species in the F.E. becomes bound to the seed calcium phosphate and if possible to promote HAP formation or other crystallization process suitable for P recovery.

Another area worth investing are design improvements to the final tank:

1. It remains to be determined what the maximum or equilibrium concentration of calcium phosphate can be retained or achieved and so the potential for reuse;
2. The analysis could refine the residence time and effect of pH of the final tank to optimise crystallization for recovery of reusable calcium phosphate. An up-flow crystal packed bed system where the final effluent could circulate in a closed loop separate from decanting for example has been reported in the literature review;
3. The extraction could also be done by:
    a. Simple batch settling after mixing the supernatant to avoid disturbing the bottom of the tank;
    b. For rapid separation a membrane or adapted membrane could be tested. The laboratory pore size filter paper 11μm used did provide the nearly same results as the 0.4 μm pore size membrane.

9.0 Conclusions

The aim of the research was to develop a more sustainable method to enhance EBPR performance compared to additions of the standard coagulants (iron and aluminium) and acetate or glucose as supplementary carbon. These have been found to be necessary to adapt the original UCT BPR process to the UK colder climate. The main conclusion from this thesis was that milk as the easily assimilible carbon source and calcium chloride as a chemical nucleation site rather than coagulation was more sustainable. Key conclusions from this research are as follows.

- Milk has been shown to be a rich source of potential substrates and nutrients when it is fermented in the anaerobic stage to release VFA.
- Anaerobically fermented milk was an ideal substrate, the experiments showed that biological activity was improved and growth rates increased;
- Optimum results were obtained using a concentration between 0.5% and 1.0% by volume typically tripling the COD (depending on the sewage strength, See annex I);
- The process milk and nucleation worked well (achieving the EU P standard above 97% of the time) when the feed concentration was below 50 mg/L;
- The use of milk and additional calcium generated a synergy which achieved better soluble P removals than the separate individual additions;
- There was no evidence to support the original hypothesis that the chelating agents present in milk persisted in WWT. The results suggested that hydrolysis of milk was delayed compared to simpler organic carbon additions, and this was also reported in the literature of other complex feedstocks. It was shown that milk, because of this and augmented nutrient profile led to a better P uptake compared to glucose. It was concluded milk was a possible model for other complex substrates.
- Membrane separation using micro-filtration (≤ 0.4 μm) had no effect on total P in the final effluent which did not consistently achieve the EU standard below 1mg/L. It may be concluded the residual P was all soluble. In contrast to filtration nucleation was able to reduce residual soluble P from enhanced biological removal (EBR) and achieve the EU standard. A minimum reduction of 97% of the soluble P from raw feed concentrations between 25 mg/L and 90 mg/L. This minimum performance was calculated without smoothing or averaging.
- The optimum soluble P removal efficiency in our experiments using milk was when the initial P concentration in the influent was between 25 mg/L and 50 mg/L;

- It was concluded that a low concentration of calcium phosphate (10mg/L) as seed in an additional post membrane process tank provided a novel and consistent method of achieving low residual P concentrations in the F.E. after EBPR. The phosphate accumulated around the calcium seed and therefore also offered the possibility of P recovery

10.0 References

Ahn, C. H., Park, J. K., and Wang, J. (2009) “Changes in Polyhydroxy-Alkanoates (PHAs) during Enhanced Biological Phosphorus Removal with Dairy Industrial Wastewater” *Journal of Environmental Engineering*, **135**: 1213 – 1220.

Akin, B.S. and Ugurlu, A. (2001). “Enhanced phosphorus removal by glucose fed sequencing batch reactor”. *Journal of Environmental Science and Health*, **36**: 1757–1766.

Akin, B.S. and Ugurlu, A. (2003). “Biological removal of carbon, nitrogen and phosphorus in a sequencing batch reactor”. *Journal of Environmental Science and Health*, **38**: 1479 – 1489.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K. and Walter, P. (2008) “Molecular Biology of the Cell” 5th Ed., Garland Science, New York (NY), USA, Chapter 2, 82 – 85.

Alkaya, E. and Demirer, G., N. (2010). “Anaerobic Acidification of Sugar-beet Processing Wastes: Effect of Operational Parameters” *Biomass and Energy*, doi:10.1016/j.biombioe.2010.08.002

Alvarez-Vazquez, H., Jefferson, B., Judd, S. J. (2004) “Membrane bioreactors vs. conventional biological treatment of landfill leachate: a brief review” *Chemical Technology and Biotechnology*, **79**: 1043 – 1049

Amiot, J., Fournier, F., Leboeuf, Y., Paquin, P. and Simpson, R. (2002) “Composition, Propriétés Physicochimiques, Valeur Nutritive, Qualité Technologique et Technique d'Analyse du Lait.” *Science et Technologie du Lait : Transformation du Lait* (Presses Internationales Polytechnique), Montreal (Canada) 1 – 73

APHA (2005) “Standard methods for the examination of water and waste water”. 21st Edition, APHA, AWWA, WPCF, Washington, D.C., USA

Aquarec, (2006) “Integrated concepts for reuse of upgraded wastewater” Guideline for quality standards for water reuse in Europe, EVK1-CT-2002-00130. Edited by Salgot, M. & Huertas, E., University of Barcelona.
[Online] URL: http://www.amk.rwth-aachen.de/fileadmin/files/Forschung/Aquarec/WP2-Aquarec-FINAL.pdf (Accessed in August 2010)

Archibald, F., Methot, M., Young, F., Paice, M., G. (2001) “A simple system to rapidly monitor activated sludge health and performance” *Water Research*, **35**: 2543 – 2553

Arros-Alileche, S., Merin, U., Daufin, G., Gesan-Guiziou, G. (2008) “The Membrane Role in an Anaerobic Membrane Bioreactor for Purification of Dairy Wastewaters: A Numerical Simulation” *Bioresource Technology*, **99**: 8237 – 8244.

Arslan-Alaton, I., Eremektar, G., Germirli-Babuna, F., Insel, G., Selcuk, H., Ozerkan, B. (2005)” Advanced oxidation of commercial textile biocides in aqueous solution: effects on acute toxicity and biomass inhibition” *Water Science Technology,* **52**: 309 – 316

Aspegren, H. (1995). “Evaluation of a High Loaded Activated Sludge Process For Biological Phosphorus Removal.” Ph.D. Thesis, Dept. of Water and Environment Engineering, Lund, Sweden: Lund University of Technology.

Assobhei, O., Chennaoui, M., Hamdani, A., Mountadar, M. (2004) “Dairy effluent characterization and treatment by coagulation-decantation” *Lait*, **84**: 317 – 328

Astaraie-Imani, M., Kapelan, Z., Fu, G., Butler, D. (2012) “Assessing the combined effects of urbanisation and climate change on the river water quality in an integrated urban wastewater system in the UK” *Journal of Environmental Management*, **112**: 1 – 9

Aulinas, M., Nieves, J., C., Cortés, U., Poch, M., (2011) “Supporting decision making in urban wastewater systems using a knowledge-based approach” *Environmental Modelling & Software*, **26**: 562 – 572

Bala Suresh and Yoshio Inoguchi, (2005). *Phosphorus and Phosphorus Chemicals*. SRI Consulting Report. http://www.sriconsulting.com/CEH/Public/Reports/760.2000/. (Accessed in January, 2012)

Balmér P. and Hultman B. (1988) “Control of phosphorus discharges: present situation and trends” *Hydrobiologia*, **170**: 305 – 319

Barat, R., Montoya, T., Borras, L., Seco, A., Ferrer, J. (2006) “Calcium effect on enhanced biological phosphorus removal” *Water Science and Technology*, **53**: 29 – 37

Barat, R., Montoya, T., Borras, L., Ferrer, J., Seco, A. (2008) “Interactions between calcium precipitation and the polyphosphate-accumulating bacteria metabolism” *Water Research*, **42**: 3415 – 3424

Barat, R., Montoya, T., Seco, A. and Ferrer, J. (2011) “Modelling biological and chemically induced precipitation of calcium phosphate in enhanced biological phosphorus removal systems” *Water Research*, **45**: 3744 – 3752

Bick, A., Plazas, J., G., Yang, F., Raveh, A., Hagin, J., Oron, G. (2009). “Immersed membrane bioreactor (IMBR) for treatment of combined domestic and dairy wastewater in an isolated farm: An exploratory case study implementing the Facet Analysis (FA)” *Desalination*, **249**: 1217 – 1222

Black, R., Bennett, S.R.G., Thomas, S.M. and Beddington, J.R. (2011) “Climate Change: Migration as adaptation” *Nature*, **478**: 447 – 449

Bond, P.L., Keller, J., Blackall, L.L. (1999). Anaerobic phosphate release from activated sludge with enhanced biological phosphorus removal: possible mechanism of intracellular pH control. *Biotechnology Bioengineering*, **63**: 507–515.

Bouhabila, E.H., Aim, R.B., Buisson, H. (2001) “Fouling Characterisation in Membrane Bioreactors.” *Separation and Purification Technology,* **22-23**: 123 – 132.

Brepols, C (2011) “Operating Large Scale Membrane Bioreactors for Municipal Wastewater Treatment.” IWA Publishing, London, UK

Brindle, K. & Stephenson, T. (1996a) “The application of membrane biological reactors for the treatment of wastewaters”. *Biotechnology Bioengineering,* **49**: 601 – 610.

Broughton, A., Pratt, S., Shilton, A. (2008) “Enhanced biological phosphorus removal for high-strength wastewater with a low rbCOD:P ratio”. *Bioresource Technology*, **99**: 1236 – 1241.

Brown, P., Ong, S.K., and Lee, Y.-W. (2011*)* "Influence of anoxic and anaerobic hydraulic retention time on biological nitrogen and phosphorus removal in a membrane bioreactor" *Desalination*, **270:** 227 – 232

Brule, G., Lenoir, J., Remeuf, F. (1997) "Le fromage : de la science à l'assurance-qualité (La Micelle de Caséine et la Coagulation du Lait). *Edited by A. and Gillis, J.-C., Eck. Lavoisier TEC & DOC*, Paris, France

Canizares, P., de Lucas, A., Rodriguez, L., and Villasenor, J. (2010) "Anaerobic uptake of different organic substrates by an enhanced biological phosphorus removal sludge". *Journal of Environmental Technology*, **21**: 397 – 405.

Cao, G., Wang, S., Peng, Y., Miao, Z. (2012) "Biological nutrient removal by applying modified four step-feed technology to treat weak wastewater" *Bioresource Technology*, **128**: 604 – 611

Carlsson, H., Aspergren, H., Lee, N. and Hilmer, A. (1997) Calcium phosphate precipitation in biological phosphorus removal systems" *Water Research*, 31: 1047 – 1055

Carvalho, G., Lemos, P.C., Oehmen, A., Reis, M.A.M., (2007) "Denitrifying phosphorus removal: linking the process performance with the microbial community structure" *Water Research*, **41**: 4383 – 4396.

Chang, I.-S., Le Clech, P., Jefferson, B., and Judd, S. (2002) "Membrane fouling in membrane bioreactors for wastewater treatment" *Environmental Engineering,* **128**: 1018 – 1029

Chuang, S.-H., Chang, W.-C., Huang, Y.-H., Tseng, C.-C. and Tai, C.-C. (2011) "Effects of different carbon supplements on phosphorus removal in low C/P ratio industrial wastewater" *Bioresource Technology*, **102**, 5461 – 5465.

Churchouse, S., (2005) "Industrial Effluent Treatment using Membrane Bioreactors". *CIWEM and IChemE Seminar*, Technical paper presented at Bridgewater (UK)

Coats, E.R., Watkins, D.L., Brinkman, C.K. and Loge, F.J. (2011) "Effect of anaerobic HRT on biological phosphorus removal and the enrichment of phosphorus accumulating organisms." *Water Environment Research*, **83**, 461 – 469.

Cooley, H., Christian-Smith, J., Gleick, P.H., Allen, L., Cohen, M. (2009) "Understanding and Reducing the Risks of Climate Change for Transboundary Waters". Pacific Institute, California (Report).

Cordell, D., Drangert, J., White, S. (2009) "The story of phosphorus: Global food security and food for thought" *Global Environmental Change*, **19**, 292 – 305

Coulibaly, L. and Agathos, S.N. (2003) "Transformation Kinetics of Mixed Polymeric Substrates under Transitory Conditions by Aspergillus Niger." *African Journal of Biotechnology*, **2**, 438 – 443.

Cross, K.J., Huq, N.L., Palamara, J.E., Perich, J.W. and Reynolds, E.C. (2005) "Physicochemical Characterization of Casein Phosphopeptide-Amorphous Calcium Phosphate Nanocomplexes." *The Journal of Biological Chemistry* **280**: 15362 – 15369

Department of Environment, Food and Rural Affairs (DEFRA) (2012). "National Policy statement for Waste Water: A framework document for planning decisions on nationally

significant waste water infrastructure. *The Stationary Office Limited*, London, United Kingdom.

De Haan, S. (1981) "Sewage sludge as a phosphate fertiliser" Commission of the European Communities: Phosphorus in sewage sludge and animal waste slurries. D. Reidel Publishing. Company, London (UK), 149 – 162.

de Haas, D.W., Wentzel, M.C., and Ekama, G.A. (2000) "The Use of Simultaneous Chemical Precipitation in Modified Activated Sludge Systems Exhibiting Biological Excess Phosphate Removal. Part 1: Literature Review." *Water SA,* **26**: 439 – 452.

De Kort, E., Minor, M., Snoeren, T., Van Hooijdonk, T. and Van Der Linden, E. (2009). "Calcium-binding capacity of organic and inorganic ortho- and polyphosphates" *Dairy Science Technology*, **89**: 283 – 299

de Kruif, C.G. (1999) "Casein Micelle Interactions." *International Dairy Journal* **9**: 183 – 188

de Lucas, A., Rodrıguez, L., Villasenor, J., Fernandez, F.J. (2007a) "Influence of industrial discharges on the performance and population of a biological nutrient removal process" *Biochemical Engineering*, **34**: 51 – 61

de Lucas, A., Rodriguez, L., Villasenor, J., Fernandez, F. J. (2007b) "Fermentation of agro-food wastewaters by activated sludge" *Water Research*, **41**: 1635 – 1644

de Ridder, M., de Jong, S., Polchar, J. and Lingermann, S. (2012) "Risks and opportunities in the global phosphate rock market" The hague Centre for Startegic Studies (HCSS), Report 17/12/12 ISBN/EAN: 978-94-91040-69-6

Demirel, B., Yenigun, O., Onay, T.T. (2005) "Anaerobic treatment of dairy wastewaters: a review". *Process Biochemistry*, **40**: 2583 – 2595

Dircks, K., Beun, J.J., van Loosdrecht, M.C.M., Heijnen, J.J., and Henze, M. (2001) "Glycogen metabolisms in aerobic mixed cultures". *Biotechnology and Bioengineering*, **73**: 85 – 94

Dominguez, D. and Gujer, W., (2006). "Evolution of a wastewater treatment plant challenges traditional design concepts" *Water Research*, **40**: 1389 – 1386

Driver, J., Lijmbach, D., Steen, I. (1999) "Why recover phosphorus for recycling, and how?" *Environmental Technology*, **20**: 651 – 662.

Duan, J. M. & Gregory, J. (2003). "Coagulation by hydrolysing metal salts." *Advances in Colloid and Interface Science*, **100:** 475 – 502.

Dworak, T., Berglund, M., Laaser, C., Strosser, P., Roussard, J., Grandmougin, B. (2007) EU Water savings potential
[Online] URL http://ec.europa.eu/environment/water/quantity/pdf/water_saving_1.pdf (Accessed in December 2012)
Eberstadt, N.N. (2012) "Looking towards 2030: A New World Coming Into Focus". Institute of Economic Affairs (IEA), 17 – 25, *Blackwell Publishing*, Oxford (United Kingdom)

Elizondo, G.M. & Lofthouse, V. (2010) "Towards a sustainable use of water at home: understanding how much, where and why?" *Journal of Sustainable Development*, **3**: 3 – 10.

Elsener, B. and Angst, U. (2007): Mechanism of electrochemical chloride removal, Corrosion Science, 49(12):4504 – 4522.

Engle, N.L., Johns, O.R., Lemos, M., Nelson, D.R. (2011) "Integrated and adaptive management of water resources: tensions, legacies, and the next best thing". *Ecology and Society*, **16**: 19, [Online] URL: http://www.ecologyandsociety.org/vol16/iss1/art19) (Accessed in October 2012)

EPA/625/R-04/108 (September 2004)
[Online] URL: http://www.epa.gov/nrmrl/wswrd/dw/smallsystems/pubs/625r04108.pdf (Accessed in August 2010)

Eurostat [Online] URL: http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Water_statistics (Accessed in October 2012).

Evren Ersahin, M., Ozgun H., Kaan Dereli, R., Ozturk, I., Roest, K., van Lier, J.B. (2012) "A review on dynamic membrane filtration: Materials, applications and future perspectives" *Bioresource Technology*, **122**: 196 – 206

Fernandez, F.J., Castro, M.C., Villasenor, J., Rodriguez, L. (2011). "Agro-Food Wastewaters as External Carbon Source to Enhance Biological Phosphorus Removal" *Chemical Engineering Journal*, **166**: 559 – 567

Filipe, C. D. M. and Daigger, G. T. (1998) "Development of a Revised Metabolic Model for the Growth of Phosphorus-Accumulating Organisms". *Water Environment Research*, **70**: 67 – 79.

Filipe, C.D.M., Daigger, G.T., Grady, Jr. C.P.L. (2001a) "A metabolic model for acetate uptake under anaerobic conditions by glycogen-accumulating organisms: stoichiometry, kinetics and effect of pH". *Biotechnology Bioengineering*, **76**: 17 – 31

Filipe C.D.M., Daigger G.T., Grady Jr. C.P.L. (2001b) "Effects of pH on the aerobic metabolism of phosphate-accumulating organisms and glycogen-accumulating organisms". Water Environmental Research, **73**: 213 – 222.

Filipe, C.D.M., Daigger, G.T., Grady, Jr. C.P.L. (2001c) "pH as a key factor in the competition between glycogen-accumulating organisms and phosphorus-accumulating organisms" *Water Environmental Research*, **73**: 223 – 232.

Filipe, C.D.M., Daigger, G.T., Grady, Jr. C.P.L. (2001d) "Stoichiometry and kinetics of acetate uptake under anaerobic conditions by an enriched culture of phosphorus-accumulating organisms at different pH" *Biotechnology Bioengineering*, **76**: 32 – 43

Fine, P., and Mingelgrin, U. (1996) "Release of Phosphorus from Waste-Activated Sludge" *Soil Science Society of America*, **60**: 505 – 511

Fleit, E. (1995). "Intracellular pH Regulation in Biological Excess Phosphorous Removal Systems" Water Research, **29**: 1787 – 1792

Flowers, J.J., He, S., Yilmaz, S., Noguera, D.R., McMahon, K.D. (2009) "Denitrification capabilities of two biological phosphorus removal sludges dominated by different 'Candidatus Accumulibacter' clades" *Environmental Microbiology Reports*, **1**: 583 – 588

Freitas, F., Temudo, M., Reis, M.A.M. (2005) “Microbial population response to changes of the operating conditions in a dynamic nutrient-removal sequencing batch reactor” *Bioprocess and Biosystems Engineering*, **28**: 199 – 209

Gaucheron, F., Le Graet, Y., Piot, M., Boyaval, E. (1996) “Determination of anions of milk by ion chromatography” *Lait,* **76**: 433 – 443

Gaval, G. and Pernelle, J.-J. (2003) “Impact of the repetition of oxygen deficiencies on the filamentous bacteria proliferation in activated sludge”. *Water Research*, **37**: 1991 – 2000

G.E. Energy & Water – Water & Process Technologies. Handbook cooling water systems, Corrosion Control-Cooling Systems (Chapter 24). Documentation accessed on-line (accessed Mai 2014) [Online] URL http://www.gewater.com/product-directory.html

Ge, S., Peng, Y., Wang, S., Guo, J., Ma, B., Zhang, L. and Cao, X. (2010) “Enhanced nutrient removal in a modified step feed process treating municipal wastewater with different inflow distribution ratios and nutrient ratios”. *Bioresource Technology,* **101**: 9012 – 9019.

Gebremariam, S.Y., Beutel, M.W., Christian, D. and Hess, T.F. (2012) “Effect of Glucose on the Performance of Enhanced Biological Phosphorus Removal Activated Sludge Enriched with Acetate.” *Bioresource Technology*, **121**: 19 – 24.

Glass, C., Silverstein, J.A.and Oh, J. (1997) “Inhibition of Denitrification in Activated Sludge by Nitrite” *Water Environmental Research*, **69**: 1086 – 1093

Glass, C. and Silverstein, J.A. (1998a) “Denitrification Kinetics of High Nitrate Concentration Water: pH Effect on Inhibition and Nitrite Accumulation” *Water Research*, **32**: 831 – 839

Golubev, S.V., Pokrovsky O.S., Savenko, V.S. (1999) “Unseeded precipitation of calcium and magnesium phosphates from modified seawater solutions” *Crystal Growth*, **205**: 354 – 360

Grappin, R. and Ribadeau-Dumas, B. (1989) “Milk Protein Analysis.” *Le Lait-Dairy Science and Technology* **69**: 357 – 416.

Guglielmi, G., Saroj,D.P., Chiarani, D. and Andreottola, G. (2007) “Sub-critical fouling in a membrane bioreactor for municipal wastewater treatment: Experimental investigation and mathematical modelling” *Water Research,* **41**: 3903 – 3914

Habraken, W.J.E.M., Tao, J., Brylka, L.J., Friedrich, H., Bertinetti, L., Schenk, A.S., Verch, A., Dmitrovic, V., Bomans, P.H.H., Frederik, P.M., Laven, J., van der Schoot, P., Aichmayer, B., De With, G., De Yoreo, J.J. and Sommerdijk, N.A.J.M. (2013) “Ion-association complexes unite classical and non-classical theories for the biomimetic nucleation of calcium phosphate” *Nature Communications*, **4**: 1507 (doi:10.1038/ncomms2490)

Harper, W.J. and Marshall, K.R. (1984) “*Surveys in Industrial Wastewater Treatment 1. Food and Allied Industries : The treatment of Wastes from Dairy Industry”.* Pitman Publishing Limited, London (UK).

Hallen, E., Lunden, A.,Tyriseva, A.-M., Westerlind, M., and Andren, A. (2010). “Composition of poorly and non-coagulating bovine milk and effect of calcium addition”. *Journal of Dairy Research*, **77**: 398 – 403.

Hassan, A.N. and Nelson, B.K. (2012) “Invited review: anaerobic fermentation of dairy food wastewater” *Dairy Science*, **95**: 6188 – 6203.

Henze, M., Gujer, W., Mino, T., Matsuo, T., Wentzel, M.C. and Marais, G.v.R (1995) “Activated Sludge Model No.2. (IAWQ Scientific and Technical Report No.3.)” London: IAWQ.

Henze M, Harremoes P, Jansen J, Arvin E (1997). “Wastewater Treatment”, 2nd Edition, *Springer-Verlag Berlin Heidelberg New-York*

Henze, M., Gujer, W., Mino, T. and van Loosdrecht, M. (2002). “Activated Sludge Models ASM1, ASM2, ASM2d and ASM3”. Edited by IWA task Group on Mathematical Modelling for Design and Operation of Biological Wastewater Treatment, IWA Publishing, Scientific and Technical reports, 2nd Ed. London (UK).

Hesselmann, R.P.X., Werlen, C., Hahn, D., van der Meer, J.R., Zehnder, A.J.B. (1999) “Enrichment, phylogenetic analysis and detection of a bacterium that performs enhanced biological phosphate removal in activated sludge” *Systematic and Applied Microbiology*, **22**: 454 – 465.

Hollender, J., van der Krol, D., Gierden, E. and Dott, W. (2002) “Effect of different carbon sources on enhanced biological phosphorus removal in a sequencing batch reactor” *World Journal of Microbiology and Biotechnology*, **18**: 355 – 360.

Holt, C. and Horne, D.S. (1996) “The hairy casein micelle: evolution of the concept and its implication for dairy technology.” *Netherlands Milk Dairy Journal* **50**: 85 – 111.

Horan, N., Manyumba, F., Wood, E. (2009) “Meeting the phosphorus consent with biological nutrient removal under UK winter conditions” *Water and Environment*, **23**: 83 – 90

Hruschka H. (1980) “Waste treatment by precipitation with lime - a cost and efficiency analysis.” Progress in Water Technology, **12**: 383 – 393

Hu, Z.R., Wentzel, M.C., Ekama, G.A. (2002) “Anoxic growth of phosphate-accumulating organisms (PAOs) in biological nutrient removal activated sludge systems” *Water Research*, **36**: 4927 – 4937

Huyskens, C. (2012) “Fouling in submerged membrane bioreactors”. PhD thesis, University of Antwerp, Belgium.

ICON – IC Consultants Ltd London (UK) (2001) Final Report. “Pollutants in urban wastewater and sewage sludge”. For the Directorate-General Environment, 194 – 204. EU publication accessed on-line in October 2012: http://ec.europa.eu/environment/waste/sludge/pdf/sludge_pollutants.pdf

Itonaga, T., Kimura, K., and Watanabe, Y. (2004) “Influence of Suspension Viscosity and Colloidal Particles on Permeability of Membrane Used in Membrane Bioreactor (MBR).” *Water Science and Technology,* **50**: 301-309

Jansen, P.M.J., Meinema K., van der Roest H.F. (2002) “Biological phosphorus removal. Manual for design and operation” Water and wastewater practitioner series: STOWA report. Ed. IWA publishing. ISBN: 1843390124. Cornwall, UK.

Jenkins, D., Fergusson, J.F. and Menard, A.B. (1971) “Chemical Process for Phosphate Removal.” *Water Research* **5**: 369 – 389.

Jeon, C.O. & Park, J.M. (2000). “Enhanced biological phosphorus removal in a sequencing batch reactor supplied with glucose as a sole carbon source”. *Water Research*, **34**: 2160 – 2170
Jimenez, B. and T. Asano, (Ed. 2008). Water Reuse: An International Survey of Current Practice, Issues and Needs. IWA Publishing, London, UK.

Jonsson, H., Baky, A., Jeppsson, U., Hellstrom, D., Karrman, E. (2005) “Composition of urine, faeces, greywater and biowaste” Urban Water, Chalmers University of Technology, Gothenburg, Sweden; Report 2005:6, CODEN:LUTEDX/(TEIE-7222)/1-49/(2005)

Judd, S. (2006) The MBR Book, Principles and applications of membrane bioreactors in water and wastewater treatment, Elsevier, Oxford, UK.

Judd, S. (2011) The MBR Book, 2nd Edition, Principles and Applications of Membrane Bioreactors for Water
and Wastewater Treatment.

Juliastuti, J.R., Baeyens, J., Creemers, C. (2003) “Inhibition of Nitrification by Heavy Metals and Organic Compounds: The ISO 9509 Test” *Environmental Engineering Science*, **20**: 79 – 90.

Kämpfer, P., Weltin, D., Hoffmeister, D. and Dott, W. (1995) “Growth requirements of filamentous bacteria isolated from bulking and scumming sludge” Water Research, **29**: 1585 – 1588.

Kanagawa, T., Kamagata, Y., Aruga, S., Kohno, T., Horn, M., and Wagner, M. (2000). “Phylogenetic Analysis of and Oligonucleotide Probe Development for Eikelboom Type 021N Filamentous Bacteria Isolated from Bulking Activated Sludge”. *Applied Environmental Microbiology,* **66**: 5043 – 5052

Kargi, F., Uygur, A. (2004*)* “Nutrient removal in a three-step sequencing batch reactor with different carbon sources” *Water Air Soil Pollution*, **156**: 71–82

Kargi, F., Uygur, A., Baskaya, H.S. (2005) “Phosphate uptake and release rates with different carbon sources in biological nutrient removal using a SBR” *Environmental Management*, **76**: 71 – 75

Kerrn-Jespersen, J.P., Henze, M. (1993) “Biological phosphorus uptake under anoxic and aerobic conditions” *Water Research*, **27**: 617 – 624

Kim, J. and Yoon, T.I. (2010) “Direct observations of membrane scale in membrane bioreactor for wastewater treatment application” Water Science & Technology, **61**: 2267 – 227.

Kim, Y. M., Park, D., Lee, D. S., Park, J. M. (2008) “Inhibitory effects of toxic compounds on nitrification process for cokes wastewater treatment” *Hazardous Materials*, **152**: 915 – 921

Kuba, T., Wachmeister, A., van Loosdrecht, M.C.M., Heijnen, J.J. (1994) “Effect of nitrate on phosphorus release in biological phosphorus removal systems” *Water Science Technology*, **30**, 263 – 269.

Kuba, T., Murnleiter, E., van Loosdrecht, M.C.M., Heijnen, J.J. (1996) “A metabolic model for biological phosphorus removal by denitrifying organisms” *Biotechnology Bioengineering*, **52**, 685 – 695.

Kuba, T., van Loosdrecht, M.C.M., Brandse, F.A., Heijnen, J.J. (1997) “Occurrence of denitrifying phosphorus removing bacteria in modified UCT-type wastewater treatment plants”. *Water Research*, **31**: 777 – 786.

Latorre, A., Malmqvist, A., Lacorte, S., Welander, T., Barcelo, D. (2007) “Evaluation of the treatment efficiencies of paper mill whitewaters in terms of organic composition and toxicity” *Environmental Pollution*, **147**, 648 – 655

Liao, B.Q., Kraemer, J.T. and Bagley, D.M. (2006). “Anaerobic membrane bioreactors: applications and research directions”. *Environmental Science & Technology*, **36**, 489 – 530

Le-Clech, P., Chen, V. and Fane, T.A.G. (2006) “Fouling in membrane bioreactors used in wastewater treatment” *Membrane Science*, **284**, 17 – 53

Le-Clech, P. (2010) “Membrane bioreactors and their uses in wastewater treatments” *Applied Microbiology Biotechnology*, **88**, 1253 – 1260

Le Graet, Y. and Brule, G. (1993). “Les Equilibres Mineraux du Lait: Influence du pH et de la Force Ionique”. *Le Lait*, **73**, 51 – 60

Lie, E. and Welander, T. (1997) “A method for determination of the readily fermentable organic fraction in municipal wastewater” *Water Research*, **31**, 1269 – 1274

Lin, H.J., Xie, K., Mahendran, B., Bagley, D.M., Leung, K.T., Liss, S.N. and Liao, B.Q. (2009) Sludge properties and their effects on membrane fouling in submerged anaerobic membrane bioreactors (SAnMBRs). *Water Research*, **43**, 3827 – 3837.

Liu, W.T., Nakamura, K., Matsuo T, Mino T. (1997) “Internal energy-based competition between polyphosphate- and glycogen-accumulating bacteria in biological phosphorus removal reactors-effect of P/C feeding ratio” *Water Research*, **31**, 1430 – 1438

Lopez-Vazquez C. M., Song Y.I., Hooijmans C. M., Brdjanovic D., Moussa M. S., Gijzen H., van Loosdrecht M. C. M. (2007). “Short-term temperature effects on the anaerobic metabolism of Glycogen Accumulating Organisms”. *Biotechnology Bioengineering*, **97**, 483 – 495

Lopez-Vazquez, C. M., Oehmen, A., Hooijmans, C. M., Brdjanovic, D., Gijzena, H. J., Yuane, Z., van Loosdrecht, M.C.M. (2009) “Modeling the PAO–GAO competition: Effects of carbon source, pH and temperature” *Water Research*, **43**, 450 – 462.

Marquet,R.,Mietton-Peuchot,M.,and Wheatley,A.D., (1999) Characterization of trickling filter effluent by particle size distribution and size exclusion chromatography Water Research **33** (6) 1415-1424.

Marshall, R.W., Nancollas, G.H., (1969). “The Kinetics of Crystal Growth of Dicalcium Phosphate Dehydrate” *Journal of Physical Chemistry*, **73**, 3838 – 3844

Martin, H.M., Hancock, J.T., Salisbury, V. and Harison, R. (2004). “Role of Xanthine Oxidoreductase as Antimicrobial Agent” *Infection and Immunity*, **72**, 4933 – 4939.

Matilainen, A., Vepsalainen, M. & Sillanpaa, M. (2010). “Natural organic matter removal by coagulation during drinking water treatment: A review.” Advances in Colloid and Interface Science, **159,** 189 – 197.

Maurer, M. and Boller, M., (1999) “Modelling of Phosphorus Precipitation in Wastewater Treatment Plants with Enhanced Biological Phosphorus Removal”, Water Science and Technology, **39**: 147 – 163.

Miqueleto, A.P., Dolosic, C.C., Pozzi, E., Foresti, E. and Zaiat, M. (2010) “Influence of carbon sources and C/N ratio on EPS production in anaerobic sequencing batch biofilm reactors for wastewater treatment”. Bioresource Technology, 101, 1324 – 1330.

Mino, T. & Satoh, H., (1994) “Metabolism of different bacterial-populations in enhanced biological phosphate removal processes” *Water Science and Technology*, **29**, 67 – 70

Mino, T., van Loosdrecht, M.C.M., Heijnen, J.J. (1998) “Microbiology and biochemistry of the enhanced biological phosphorus removal process” *Water Research*, **32**, 3193 – 3207.

Melin T., Jefferson B., Bixio D., Thoeye C., De Wilde W., De Koning J., van der Graaf J., T. Wintgens (2006) “Membrane bioreactor technology for wastewater treatment and reuse”. *Desalination*, 187: 271 – 282

Meng, F., Zhang, H., Yang, F., Li, Y., Xiao, J. and Zhang, X. (2006a) “Effect of Filamentous Bacteria on Membrane Fouling in Submerged Membrane Bioreactor.” *Journal of Membrane Science* **272**: 161 – 168.

Molinos-Senante, M., Hernández-Sancho, F. and Sala-Garrido, R. (2011) “Cost-benefit analysis of water-reuse projects for environmental purposes: A case study for Spanish wastewater treatment plants” *Environmental Management*, **92**: 3091 – 3097.

Montoya, T., Borrás, L., Aguado, D., Ferrer, J., and Seco, A. (2008) “Detection and prevention od enhanced biological phosphorus removal deterioration caused by zoogloea overabundance”. *Environmental Technology*, **29**: 35 – 42.

Moss, R.H., Edmonds, J.A., Hibbard, K.A., Manning, M.R., Rose, S.K., Van Vuuren, D.P., Carter, T.R., Emori, S., Kainuma, M., Kram, T., Meehl, G.A., Mitchell, J.F.B., Nakicenovic, N., Riahi, K., Smith, S.J., Stouffer, R.J., Thomson, A.M., Weyant, J.P., Wilbanks, T.J. (2010). “The next generation of scenarios for climate change research and assessment”. *Nature*, **463**: 747 – 756.

Mulkerrins, D., Dobson, A.D.W., Colleran, E. (2004). “Parameters affecting biological phosphate removal from wastewaters” *Environment International*, **30**: 249 – 259

Naessens, W., Maere, T. and Nopens, I. (2012a) “Critical review of membrane bioreactor models – Part 1: Biokinetic and filtration models” *Bioresource Technology*, **122**: 95 – 106

Naessens, W., Maere, T., Ratkovich, N., Vedantam, S. and Nopens, I. (2012b) “Critical review of membrane bioreactor models – Part 2: Hydrodynamic and integrated models” *Bioresource Technology,* **122**: 107 – 118

Neethling, J.B., Bakke, B., Benisch, M., Gu, A., Stephens, H., Stensel, H.D. and Moore, R. (2005). Factors Influencing the Reliability of Enhanced Biological Phosphorus Removal. Final Report, Water Environment Research Foundation, Alexandria, VA.

National Intelligence Council (NIC) (2012) “Global Trends 2030: Alternative Worlds” Office of the Director of National Intelligence, Report NIC 2012-001. [On Line] URL: http://fr.scribd.com/doc/115962650/Global-Trends-2030-Alternative-Worlds (Accessed in April 2013)

Novotny, V. (2010). “Water and Energy in the cities of the Future – Achieving Net Zero Carbon and Pollution Emissions Footprint” IWA World Water Congress and Exhibition, Montreal, Canada.

Oehmen, A., Vives, M.T., Lu, H., Yuan, Z., Keller, J. (2005a) “The effect of pH on the competition between polyphosphate-accumulating organisms and glycogen-accumulating organisms” *Water Research*, **39**: 3727 – 3737

Oehmen, A., Zeng, R.J., Yuan, Z., Keller, J. (2005b) “Anaerobic metabolism of propionate by polyphosphate-accumulating organisms in enhanced biological phosphorus removal systems” *Biotechnology Bioengineering*, **91**: 43 – 53

Oehmen, A., Yuan, Z., Blackall, L.L., Keller, J. (2005c) “Comparison of acetate and propionate uptake by polyphosphate accumulating organisms and glycogen accumulating organisms” *Biotechnology Bioengineering*, **91**: 162 – 168.

Oehmen, A., Saunders, A.M., Vives, M.T., Yuan, Z., Keller, J. (2006a) “Competition between polyphosphate and glycogen accumulating organisms in enhanced biological phosphorus removal systems with acetate and propionate as carbon sources” *Biotechnology*, **123**: 22 – 32

Oehmen, A., Zeng, R.J., Saunders, A.M., Blackall, L.L., Keller, J., Yuan, Z. (2006b) “Anaerobic and aerobic metabolism of glycogen accumulating organisms selected with propionate as the sole carbon source” *Microbiology*, **152**: 2767 – 2778.

Oehmen, A., Lemos, P.C., Carvalho, G., Yuan, Z., Keller, J., Blackall, L.L., Reis, M.A.M. (2007a) “Advances in enhanced biological phosphorus removal: from micro to macro scale” *Water Research*, **41**: 2271 – 2300.

Oehmen, A., Zeng, R.J., Keller, J., Yuan, Z. (2007b) “Modeling the aerobic metabolism of polyphosphate-accumulating organisms enriched with propionate as a carbon source” *Water Environmental Research*, **79:** 2477 – 2486

Ohata, M. and Furuta, N. (1998) “Evaluation of the detection capability of a high-power microwave-induced plasma for both atomic emission and mass spectrometry” *Analytical Atomic Spectrometry*, **13**: 447 – 453

Oki, T. and Kanae, S. (2006) “Global hydrological cycles and world water resources” *Science*, **313**: 1068 – 1072.

Oller, I., Malato, S., Sánchez-Pérez, J. A. (2011) “Combination of advanced oxidation processes and biological treatments for wastewater decontamination—A review” *Science of the Total Environment*, **409**: 4141 – 4166

Palmowski, L., Veltmann K, Pinnekamp, J. and Weber, M. (2011). “Energy optimisation of Large Scale Membrane Bioreactors Using the Example of the WWTP Seelscheid.” In Lazarova,V., Choo, K.H., Cornel, P. (Eds.): Water-Energy-Interactions of Water Reuse, IWA Publishing pp139 – 148

Palmquist, H. and Hanæus, J. (2005) “Hazardous substances in separately collected grey- and blackwater from ordinary Swedish households” *Science of the Total Environment*, **348:** 151 – 163

Panouillé, M., Durand, D., Nicolai, T., Larquet, E. and Boisset, N. (2005) “Aggregation and gelation of micellar casein particles” *Colloids and Interface Science,* **287**: 85 – 93
Park, Y.W. and Haenlein, G.F.W. (2013) “Milk and Dairy Products in Human Nutrition” 1st Ed. John Wiley & Sons, Ltd. (UK)

Peng, Y.Z. and Ge, S.J. (2011) “Enhanced nutrient removal in three types of step feeding process from municipal wastewater”. *Bioresource and Technology*, **102**: 6405-6413

Peterson, G.D., Cumming, G.S., Carpenter, S.R. (2003). “Scenario planning: a tool for conservation in an uncertain world”. *Conservation Biology*, **17**: 358 – 366.

Pinnekamp, J. and Friedrich, H. (2006) “Membrane Technology for Waste Water Treatment” *FiW Publishing*, Aachen, Germany.

Pinnekamp, J. and Friedrich, H. (2009) “Municipal Water and Waste Management: Membrane Technology for Waste Water Treatment”. *FiW Publishing*, Aachen, Germany.

Pouliot, Y., Landry, J., Giasson, J. (1991). “Induction of calcium phosphate precipitation in sweet whey permeate”. *Le Lait*, **71**: 313 – 320

Puig S, Corominas LI, Balaguer MD, Colprim J (2007) Biological nutrient removal by applying SBR technology in small wastewater treatment plants: carbon source and C/N/P ratio effects. *Water Science Technology*, **55**: 135-141.

Randall, A.A., Benefield, L.D., Hill, W.E. (1997) “Induction of phosphorus removal in an enhanced biological phosphorus removal bacterial population” *Water Research*, **31**: 2869 – 2877

Randall, A.A. (2012). “Contrast of Volatile Fatty Acid Driven and Inorganic Acid or Base Driven Phosphorus Release and Uptake in Enhanced Biological Phosphorus Removal” *Water Environment Research*, **84**: 305 – 312

Raskin, P., Gleick, P. H., Kirshen, P., Pontius, R. G. Jr. and Strzepek, K., (1997). “Comprehensive assessment of the freshwater resources of the world” *Stockholm Environmental Institute*, Sweden. Document prepared for the fifth session of the United Nations Commission on Sustainable Development, 1997.

Ren, S., (2004) “Assessing wastewater toxicity to activated sludge: recent research and developments” *Environmental International*, **30**, 1151 – 1164

Rensink, J.H., van der Ven, J., van Pamelen, G., Fedder, F., Majoor, E. (1997) “The modified Renphosystem: a high biological nutrient removal system”. *Water Science Technology*, **35**, 137– 146

Saito, T., Brdjanovic, D., van Loosdrecht, M.C.M. (2004) “Effect of nitrite on phosphate uptake by phosphate accumulating organisms” *Water Research*, **38**, 3760 – 3768.

Schmidt, D.G., Both, P., Visser, S., Slangen, K.J., Van Rooijen, P.J. (1987) “Studies on the precipitation of calcium phosphate : Experiments in the pH range 7.3 to 5.8 in the presence of additives”. *Netherlands Milk Dairy Journal*, **41**, 121 – 136.

Schröder, J. J., Smit, A. L. Cordell, D. and Rosemarin. A. (2011) “Improved Phosphorus Use Efficiency in Agriculture: A Key Requirement for Its Sustainable Use.” *Chemosphere,* **84**, 822 – 831.

Schuler, A.J., Jenkins, D. (2002) “Effects of pH on enhanced biological phosphorus removal metabolisms” *Water Science Technology*, **46**, 171 – 178.

Schuler, A.J., Jenkins, D. (2003) “Enhanced biological phosphorus removal from wastewater by biomass with different phosphorus contents, Part 1: Experimental results and comparison with metabolic models” *Water Environmental Research*, **75**, 485 – 498.

Seviour, R.J., Mino, T., Onuki, M. (2003) “The microbiology of biological phosphorus removal in activated sludge systems” *FEMS Microbiology Reviews*, **27**, 99 – 127.

Seckler, M.M., Bruisma, O.S.L. and van Rosmalen, G.M., 1996a, “Calcium phosphate precipitation in a fluidized bed in relation to process conditions: a black box approach”. *Water Resources*, **30**: 1677 – 1685.

Silva, A., Carvalho, G., Oehmen, A., Lousada-Ferreira, M., Nieuwenhuijzen, A., Reis, M., Crespo, M. (2012) “Microbial population analysis of nutrient removal-related organisms in membrane bioreactors” *Applied Microbiology and Biotechnology*, **93**, 2171 – 2180.

Smith, A. L., Stadler, L. B., Love, N. G., Skerlos, S. J., Raskin, L. (2012) “Perspectives on anaerobic membrane bioreactor treatment of domestic wastewater: A critical review” *Bioresource Technology*, **122**, 149 – 159

Smolders, G.J.F., van der Meij, J., van Loosdrecht, M.C.M., Heijnen, J.J. (1994a) “Model of the anaerobic metabolism of the biological phosphorus removal process: stoichiometry and pH influence”. *Biotechnology Bioengineering*, **43**, 461 – 470.

Smolders, G.J.F., van der Meij, J., van Loosdrecht, M.C.M., Heijnen, J.J. (1995) “A structured metabolic model for anaerobic and aerobic stoichiometry and kinetics of the biological phosphorus removal process”. *Biotechnology Bioengineering*, **47**, 277 – 287.

SOER 2010 water ressources: quantity and flows (EEA, 2010). Kristensen, P. (EEA lead author**,** coordinator). EEA contributors, Werner, B., Collins, R., Jacobsen, B., Uhel, R. and Wehrli, A. EEA's European Topic Centre on Water, Koutiva, I. and Kossida, M. (NTUA). EEA, Copenhagen, 2010.
http://www.eea.europa.eu/soer/europe/water-resources-quantity-and-flows/at_download/file
(accessed through the Europa server www.europa.eu 19 October 2012).

Song, Y., Hahn, H.H. and Hoffmann E. (2002) “Effects of solution conditions on the precipitation of phosphate for recovery, a thermodynamic evaluation”. *Chemosphere*, **48**: 1029 – 1035.

Spellman, F.R. (2008) “Handbook of Water and Wastewater Treatment Plant Operations” Second Edition, CRC Press (Taylor & Francis Group). Florida (USA), 735 – 745

Strzepek, K., Boehlert, B. (2010) “Competition for water for the food system”. *Philosophical Transactions of the Royal Society of Biological Sciences*, **365**, 2927 – 2940.

Sudiana, I.M., Mino, T., Satoh, H., Nakamura, K., Matsuo, T. (1999) “Metabolism of enhanced biological phosphorus removal and non-enhanced biological phosphorus removal sludge with acetate and glucose as carbon source”. *Water Science Technology*, **39**, 29 – 35

Tchobanoglous, G., Burton, F.L. and Stensel, H.D. (2003). Metcalf & Eddy, Inc.'s “Wastewater Engineering: Treatment, Disposal, and Reuse”, 4th Edition. McGraw-Hill, Inc. (Metcalf and Eddy), New York (USA).

Tiidus, P.M., Tupling, A.R., Houston, M.E. (2012) “Biochemistry Primer for Exercise Science” 4th Ed. Human Kinetics Publishers, Champaign (IL), USA, 139 - 143

Tong, K., Zhang, Y., Chu, P.K. (2013) “Evaluation of calcium chloride for synergistic demulsification of super heavy oil wastewater” *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **419**, 46 – 52

Trivedi, H.K. (2004) “Flat-plate microfiltration membrane bioreactor designed for ultimate nutrient removal (UNR™)” *Water Environment Federation*. Technical paper presented at *WEFTEC® 2004*. Austin, Texas (USA)

Ubukata, Y. (1997). “Kinetics of Polymeric Substrate (Dextrin or Peptone) Removal by Activated Sludge: Hydrolysis of Polymers to Monomers is the Rate-Determining Step.” *Water Science and Technology*, **36**, 159 – 167.

Uchino, K., Saito, T., Gebauer, B. and Jendrossec, D. (2007) “Isolated Poly (3-hydroxybutyrate) (PHB) Granules are complex Bacterial Organelles Catalysing Formation of PHB from acetyl Coenzyme A (CoA) and Degradation of PHB to Acetyl-CoA” *Journal of Bacteriology*, **189**, 8250 – 8256.

Vaiopoulou, E., Melidis, P. and Aivasidis, A. (2007) “Growth of filamentous bacteria in an enhanced biological phosphorus removal system”. *Desalination*, **213**, 288 – 296.

Van der Houwen, J.A.M., Cressey, G., Cressey, B.A., Valsami-Jones, E. (2003) “The effect of organic ligands on the crystallinity of calcium phosphate” *Journal of Crystal Growth*, **249**: 572 – 583

Van Kemenade, M.J.J.M. and De Bruyn, P.L. (1987) “A kinetic study of precipitation from supersaturated calcium phosphate solutions” *Colloid Interface Science*, **118**: 564 – 585.

Van Kemenade, M., J., J., M., and de Bruyn P., L. (1989a) “The influence of casein on the kinetics of hydroxyapatite precipitation”. *Journal of Colloidal and Interface Science*, **129**: 1 – 14.

van Voorthuizen, E., Zwijnenburg, A., van der Meer, W., Temmink, H., (2008) “Biological black water treatment combined with membrane separation” *Water Research*, **42:** 4334 – 4340

Van Vuuren, D., Bouwman, A., Beusen, A. (2010). “Phosphorus demand for the 1970 – 2100 period: a scenario analysis of resource depletion” *Global Environmental Change*, **20:** 428 – 439

Walstra, P. and Jenness, R. (1984) “Dairy Chemistry and Physics” John Wiley and Sons, New-York

Wang, J., Baltzis, B. and Lewandowski, G. (1995) “Fundamental denitrification kinetic studies with Pseudomonas denitrificans” *Biotechnology and Bioengineering*, **47**: 27 – 41

Wang, Y., Peng, Y., Li, T., Ozaki, M., Takigawa, A., Wang, S. (2004) “Phosphorus removal under anoxic conditions in a continuous-flow A_2N two-sludge process” *Water Science Technology*, **50**: 37 – 44.

Wang, Y., Jiang, F., Zhang, Z., Xing, M., Lu, Z., Wu, M., Yang, J., Peng, Y. (2010) “The long-term effect of carbon source on the competition between polyphosphorus accumulating organisms and glycogen accumulating organism in a continuous plug-flow anaerobic/aerobic (A/O) process”. *Bioresource Technology*, **101**: 98 – 104.

Wang, Z, Liu, B., Liu, Y.D. and Wan, F (2013). “Evaluation of biological removal efficiency in a UCT process treating municipal wastewater during start-up stage” *Environmental Biology.,* **34**, 459 – 464.

Wentzel, M.C., Ekama, G.A. and Marais G.v.R. (1991) “Kinetics of nitrification-denitrification biological phosphorus removal systems - A review” *Water Science Technology,* **23**, 555 – 565

Wentzel, M.C., Ekama, G.A. (1997), “Principles in the design of single sludge activated-sludge systems for biological removal of carbon, nitrogen and phosphorus” Water Environment Research, **69**: 1222 – 1231.

Whang, L.M., Park, J.K. (2006) “Competition between polyphosphate- and glycogen-accumulating organisms in enhanced biological phosphorus removal systems: effect of temperature and sludge age” *Water Environmental Research*, **78**, 4 – 11.

Wilemena, A., Ozkanb, A. and Berberoglu, H., (2012) “Rheological properties of algae slurries for minimizing harvesting energy requirements in biofuel production”. Bioresource Technology, **104**: 432 – 439.

Williams, R.P.W., D’ath, L. and Augustin, M.A. (2005) “Production of Calcium – Fortified Milk Powders Using Soluble Calcium Salts”. *Le Lait*, **85**, 369 – 381.

Woertz, I., Feffer, A., Lundquist, T., and Nelson, Y. (2009) ”Algae Grown on Dairy and Municipal Wastewater for Simultaneous Nutrient Removal and Lipid Production for Biofuel Feedstock.” *Environmental Engineering*, **135**: 1115 – 1122.

Wong, M.T., Mino, T., Seviour, R.J., Onuki, M., Liu, W.T. (2005) “In situ identification and characterization of the microbial community structure of full-scale enhanced biological phosphorous removal plants in Japan” *Water Research*, **39**, 2901 – 2914.

Yang, W.B., Cicek, N., Ilg, J. (2006) “State-of-the-art of membrane bioreactors: Worldwide research and commercial applications in North America”. *Membrane Science* 270, 201 – 211.

Yeoman, S., Stephenson, T., Lester, J.N. and Perry, R. (1988) “The removal of phosphorus during wastewater treatment. A review.” *Environmental Pollution,* **49**, 183 – 233.

Zeng, W., Li, L., Yang, Y.-Y., Wang, X.-D., Peng, Y.,-Z. (2011) “Denitrifying phosphorus removal and impact of nitrite accumulation on phosphorus removal in a continuous anaerobic–anoxic–aerobic (A^2O) process treating domestic wastewater” *Enzyme and Microbial Technology*, **48**, 134 – 142.

Zhang M, Tay J.H., Qian Y. and Gu X.S. (1988) “Coke plant wastewater treatment by fixed biofilm system for COD and NH3-N removal” Water Research, 32, 519 – 527.

Zhang, Z., Tan, Y., Zhong, M. (2011) “Defluorination of wastewater by calcium chloride modified natural zeolite” Desalination, **276**, 246 – 252.

Zhu, G.B., Peng, Y.Z., Li, B.K., Guo, J.H., Yang, Q., Wang, S.Y. (2008) “Biological removal of nitrogen from wastewater” *Reviews of Environmental Contamination and Toxicology*, **192**, 159 – 195.

Annex

Annex I: TOC and COD

		PHASE I (1%)								
		Measure 1	Measure 2	**COD Feed(mg/l)**	Measure 1	Measure 2	**COD FE (mg/l)**	T.C.	I.C.	**T.O.C. FE**
23/05/2011	LINE A	1498	1571	**1535**	206	192	**199**	44.03	31.25	**12.78**
	LINE B	1960	1399	**1680**	191	194	**193**	41.11	26.48	**14.63**
24/05/2011	LINE A							59.30	47.94	**11.36**
	LINE B							62.89	54.64	**8.25**
25/05/2011	LINE A							62.14	52.96	**9.18**
	LINE B							68.64	58.52	**10.12**
26/05/2011	LINE A							72.25	62.68	**9.57**
	LINE B							66.86	60.41	**6.45**
27/05/2011	LINE A							76.35	68.97	**7.38**
	LINE B							71.78	66.43	**5.35**
30/05/2011	LINE A							105.8	97.6	**8.20**
	LINE B							99.65	83.7	**15.95**
31/05/2011	LINE A							130.6	113.8	**16.80**
	LINE B							93.00	77.09	**15.91**
01/06/2011	LINE A	1411	1498	**1455**				113.00	106.40	**6.60**
	LINE B	1288	1237	**1263**				99.17	87.99	**11.18**
02/06/2011	LINE A							123.60	109.50	**14.10**
	LINE B							105.80	93.73	**12.07**
03/06/2011	LINE A							129.60	117.60	**12.00**
	LINE B							111.05	98.78	**12.27**
06/06/2011	LINE A	0	0	**0**	110	108	**109**	111.50	97.83	**13.67**
	LINE B	0	0	**0**	75	81	**78**	110.00	95.26	**14.74**
07/06/2011	LINE A							96.29	81.89	**14.40**
	LINE B							103.00	90.89	**12.11**

PHASE I (1%)

		Measure 1	Measure 2	COD Feed(mg/l)	Measure 1	Measure 2	COD FE (mg/l)	T.C.	I.C.	T.O.C. FE
08/06/2011	LINE A							93.32	76.00	**17.32**
	LINE B							95.03	86.81	**8.22**
09/06/2011	LINE A							91.78	81.39	**10.39**
	LINE B							99.49	89.76	**9.73**
10/06/2011	LINE A							90.72	76.34	**14.38**
	LINE B							97.82	86.1	**11.72**

PHASE I (2.5%)

		Measure 1	Measure 2	COD Feed(mg/l)	Measure 1	Measure 2	COD FE (mg/l)	T.C.	I.C.	T.O.C. FE
13/06/2011	LINE A	3892	3778	**3835**	330	324	**327**	106.60	86.18	**20.42**
	LINE B	1078	1102	**1090**	97	101	**99**	85.20	78.32	**6.88**
14/06/2011	LINE A							123.3	101.9	**21.40**
	LINE B							96.44	85.35	**11.09**
15/06/2011	LINE A							148.80	123.20	**25.60**
	LINE B							94.07	87.22	**6.85**
16/06/2011	LINE A							142.60	124.30	**18.30**
	LINE B							88.24	78.93	**9.31**
17/06/2011	LINE A							134.00	115.30	**18.70**
	LINE B							91.12	75.93	**15.19**
20/06/2011	LINE A							144.35	122.55	**21.80**
	LINE B							81.22	69.07	**12.15**
21/06/2011	LINE A							152.10	131.20	**20.90**
	LINE B							86.64	75.65	**10.99**
22/06/2011	LINE A							152.80	137.50	**15.30**
	LINE B							83.83	74.10	**9.73**

PHASE I (2.5%)

		Measure 1	Measure 2	**COD Feed(mg/l)**	Measure 1	Measure 2	**COD FE (mg/l)**	T.C.	I.C.	**T.O.C. FE**
23/06/2011	LINE A							145.55	126.90	**18.65**
	LINE B							83.09	74.19	**8.90**
24/06/2011	LINE A							144.00	124.30	**19.7**
	LINE B							81.82	73.970	**7.85**
27/06/2011	LINE A							180.20	157.40	**22.8**
	LINE B							89.15	82.59	**6.56**
28/06/2011	LINE A							148.60	134.50	**14.1**
	LINE B							81.33	72.72	**8.61**
29/06/2011	LINE A							141.20	130.90	**10.3**
	LINE B							86.94	79.75	**7.19**
30/06/2011	LINE A							140.10	128.05	**12.05**
	LINE B							78.31	70.26	**8.05**

					PHASE II					
		T.C.	I.C.	**T.O.C. AN**	T.C.	I.C.	**T.O.C. AX**	T.C.	I.C.	**T.O.C. FE**
01/07/2011	LINE A			**0**			**0**	93.39	86.63	**6.76**
	LINE B			**0**			**0**	88.21	81.54	**6.67**
02/07/2011	LINE A							102.50	95.17	**7.33**
	LINE B							103.05	95.97	**7.08**
03/07/2011	LINE A							109.40	100.20	**9.20**
	LINE B							120.20	106.30	**13.90**
04/07/2011	LINE A							121.90	108.30	**13.60**
	LINE B							138.20	130.30	**7.90**
05/07/2011	LINE A							140.10	125.70	**14.40**
	LINE B							110.40	100.40	**10.00**
06/07/2011	LINE A							106.00	98.12	**7.88**
	LINE B							125.85	113.50	**12.35**
07/07/2011	LINE A							136.10	125.00	**11.10**
	LINE B							120.10	112.00	**8.10**
08/07/2011	LINE A							145.30	127.30	**18.00**
	LINE B							116.40	103.40	**13.00**
09/07/2011	LINE A							141.00	130.40	**10.60**
	LINE B							113.10	101.40	**11.70**
10/07/2011	LINE A							139.50	130.30	**9.20**
	LINE B							107.90	99.17	**8.73**
11/07/2011	LINE A							138.80	130.70	**8.10**
	LINE B							110.20	100.55	**9.65**
12/07/2011	LINE A							119.20	113.70	**5.50**
	LINE B							103.70	93.90	**9.80**
13/07/2011	LINE A							107.30	99.78	**7.52**
	LINE B							99.31	86.35	**12.96**
14/07/2011	LINE A							99.62	86.11	**13.51**
	LINE B							93.28	82.62	**10.66**

15/07/2011	LINE A							100.40	93.68	**6.72**
	LINE B							93.76	79.04	**14.72**

PHASE III (1/2)

		T.C.	I.C.	**T.O.C. FEED**	T.C.	I.C.	**T.O.C. ANAEROB**	T.C.	I.C.	**T.O.C. FE**
10/10/2011	LINE A			**0.00**	156.8	96.3	**60.50**	47.07	25.08	**21.99**
	LINE B			**0.00**	70.15	45.11	**25.04**	21.08	7.79	**13.29**
11/10/2011	LINE A			**0.00**	165.3	98.88	**66.42**	46.61	36.18	**10.43**
	LINE B			**0.00**	78.83	66.38	**12.45**	11.52	1.91	**9.61**
13/10/2011	LINE A			**0.00**	148.1	94.32	**53.78**	47.63	37.84	**9.79**
	LINE B			**0.00**	69.17	41.73	**27.44**	15.73	4.41	**11.32**
14/10/2011	LINE A			**0.00**	163.1	108.8	**54.30**	41.26	30.84	**10.42**
	LINE B			**0.00**	81.53	42.77	**38.76**	14.72	3.32	**11.40**
21/10/2011	LINE A			**0.00**	177.4	114.4	**63.00**	40.94	30.08	**10.86**
	LINE B			**0.00**	101.8	64.79	**37.01**	21.67	7.23	**14.44**
24/10/2011	LINE A			**0.00**	152.4	101.8	**50.60**	50.93	38.56	**12.37**
	LINE B			**0.00**	121.4	85.48	**35.92**	22.19	9.89	**12.30**
25/10/2011	LINE A			**0.00**	163.3	98.7	**64.60**	48.12	38.90	**9.22**
	LINE B			**0.00**	121.3	76.26	**45.04**	15.66	3.78	**11.88**
26/10/2011	LINE A			**0.00**	122.9	73.69	**49.21**	51.35	40.13	**11.22**
	LINE B			**0.00**	87.86	62.81	**25.05**	16.68	4.03	**12.65**
27/10/2011	LINE A			**0.00**	104.5	54.56	**49.94**	51.22	34.16	**17.06**
	LINE B			**0.00**	65.11	47.28	**17.83**	16.76	5.22	**11.54**
28/10/2011	LINE A			**0.00**	103.7	56.2	**47.50**	45.11	34.62	**10.49**
	LINE B			**0.00**	85.53	49.89	**35.64**	8.93	1.11	**7.82**
31/10/2011	LINE A			**0.00**	141.8	92.3	**49.50**	40.80	30.58	**10.22**
	LINE B			**0.00**	91.62	58.66	**32.96**	10.59	2.66	**7.93**
01/11/2011	LINE A			**0.00**	192.7	79.21	**113.49**	39.44	26.07	**13.37**
	LINE B			**0.00**	133.6	97.33	**36.27**	13.78	1.29	**12.49**
02/11/2011	LINE A			**0.00**	182.1	81.92	**100.18**	43.30	38.82	**4.48**
	LINE B			**0.00**	110.5	86.06	**24.44**	11.42	2.98	**8.44**

PHASE III (1/2)

		T.C.	I.C.	**T.O.C. FEED**	T.C.	I.C.	**T.O.C. ANAEROB**	T.C.	I.C.	**T.O.C. FE**
03/11/2011	LINE A	116.5	92.68	**23.82**	181.4	66.93	**114.47**	43.90	39.09	**4.81**
	LINE B	116.5	92.68	**23.82**	105.4	85.37	**20.03**	11.09	4.19	**6.90**
04/11/2011	LINE A	110.8	77.26	**33.54**	195.1	62.27	**132.83**	52.68	45.97	**6.71**
	LINE B	110.8	77.26	**33.54**	107.7	84.6	**23.10**	19.32	10.16	**9.16**
05/11/2011	LINE A	107.8	61.75	**46.05**	212.2	52.62	**159.58**	43.67	35.75	**7.92**
	LINE B	107.8	61.75	**46.05**	107.2	82.71	**24.49**	12.83	2.93	**9.90**
06/11/2011	LINE A			**0.00**	164.3	50.11	**114.19**	40.22	25.37	**14.85**
	LINE B			**0.00**	84.3	40.78	**43.52**	11.67	2.73	**8.94**
07/11/2011	LINE A	88.69	54.15	**34.54**	117.7	49.88	**67.82**	34.03	25.41	**8.62**
	LINE B	88.69	54.15	**34.54**	75.8	51.77	**24.03**	10.39	1.12	**9.27**

PHASE III (2/2)

		T.C.	I.C.	**T.O.C. FEED**	T.C.	I.C.	**T.O.C. ANAEROB**	T.C.	I.C.	**T.O.C. FE**
08/11/2011	LINE A			**0.00**	176.3	55.67	**120.63**	31.63	23.12	**8.51**
	LINE B			**0.00**	79.64	56.15	**23.49**	9.76	1.27	**8.49**
09/11/2011	LINE A	84.54	46.19	**38.35**	226.30	33.20	**193.10**	31.33	23.65	**7.68**
	LINE B	84.54	46.19	**38.35**	76.21	53.78	**22.43**	9.77	1.03	**8.74**
10/11/2011	LINE A			**0.00**	234.9	35.21	**199.69**	27.83	21.29	**6.54**
	LINE B			**0.00**	76.98	56.02	**20.96**	9.29	1.62	**7.67**
11/11/2011	LINE A	52.52	30.51	**22.01**	178.9	38.86	**140.04**	27.75	18.51	**9.24**
	LINE B	52.52	30.51	**22.01**	61.2	39.85	**21.35**	9.96	0.97	**8.99**
14/11/2011	LINE A	111.5	57.95	**53.55**	233.3	61.01	**172.29**	28.47	25.30	**3.17**
	LINE B	111.5	57.95	**53.55**	67.47	45.54	**21.93**	8.97	1.26	**7.71**
15/11/2011	LINE A			**0.00**	253.5	57.12	**196.38**	29.84	21.72	**8.12**
	LINE B			**0.00**	62.8	42.61	**20.19**	9.33	0.28	**9.05**

PHASE III (2/2)

		T.C.	I.C.	T.O.C. FEED	T.C.	I.C.	T.O.C. ANAEROB	T.C.	I.C.	T.O.C. FE
16/11/2011	LINE A	133.5	68.59	**64.91**	201.1	69.41	**131.69**	30.22	22.67	**7.55**
	LINE B	133.5	68.59	**64.91**	268.77	86.8	**181.97**	29.80	20.44	**9.36**
17/11/2011	LINE A			**0.00**	198.2	70.51	**127.69**	33.67	24.81	**8.86**
	LINE B			**0.00**	265.03	83.05	**181.98**	29.01	22.03	**6.98**
18/11/2011	LINE A	137.1	79.07	**58.03**	195.9	72.21	**123.69**	34.36	27.90	**6.46**
	LINE B	137.1	79.07	**58.03**	269.75	82.05	**187.70**	30.57	22.57	**8.00**
21/11/2011	LINE A	127.1	66.25	**60.85**	187.5	72.36	**115.14**	31.41	23.26	**8.15**
	LINE B	127.1	66.25	**60.85**	128.2	60.57	**67.63**	22.20	17.09	**5.11**
22/11/2011	LINE A	97.87	66.05	**31.82**	152.7	67.97	**84.73**	28.53	18.00	**10.53**
	LINE B	97.87	66.05	**31.82**	86.75	55.68	**31.07**	20.84	17.85	**2.99**
23/11/2011	LINE A			**0.00**	157.2	65.01	**92.19**	23.75	16.00	**7.75**
	LINE B			**0.00**	86.9	57.69	**29.21**	22.01	16.14	**5.87**
24/11/2011	LINE A			**0.00**	181.0	78.39	**102.61**	19.95	9.37	**10.58**
	LINE B			**0.00**	89.8	54.68	**35.12**	12.85	8.34	**4.51**
25/11/2011	LINE A			**0.00**	227.7	62.11	**165.59**	31.32	20.48	**10.84**
	LINE B			**0.00**	87.9	52.51	**35.39**	11.45	9.54	**1.91**
28/11/2011	LINE A	101.4	67.24	**34.16**	213.7	74.09	**139.61**	36.26	22.63	**13.63**
	LINE B	101.4	67.24	**34.16**	89.1	53.47	**35.63**	14.50	11.49	**3.01**
29/11/2011	LINE A	137.8	73.27	**64.53**	202.8	72.89	**129.91**	29.34	22.70	**6.64**
	LINE B	137.8	73.27	**64.53**	123.8	61.15	**62.65**	10.52	8.42	**2.10**
30/12/2011	LINE A			**0.00**	201.9	72.21	**129.69**	30.62	21.32	**9.30**
	LINE B			**0.00**	125.2	66.5	**58.70**	9.42	7.75	**1.67**
01/12/2011	LINE A	118.9	76.7	**42.20**	200.5	84.65	**115.85**	29.83	24.73	**5.10**
	LINE B	118.9	76.7	**42.20**	102.8	63.63	**39.17**	9.58	8.13	**1.45**
02/12/2011	LINE A			**0.00**	201.2	84.62	**116.58**	32.34	23.26	**9.08**
	LINE B			**0.00**	112.7	67.85	**44.85**	12.84	10.64	**2.20**
05/12/2011	LINE A	113.3	76.45	**36.85**	183.3	87.38	**95.92**	37.78	30.66	**7.12**
	LINE B	113.3	76.45	**36.85**	97.95	66.13	**31.82**	13.14	11.23	**1.91**

Annex II: TOC Continuous test

		T.C.	I.C.	T.O.C. FEED	T.C.	I.C.	T.O.C. F.E.	T.C.	I.C.	T.O.C. AX	T.C.	I.C.	T.O.C. AN
20/06/2012	1			**0.00**	79.11	77.48	**1.63**						
21/06/2012	2			**0.00**	80.03	74.93	**5.10**						
22/06/2012	3			**0.00**	82.11	70.24	**11.87**						
25/06/2012	4			**0.00**	77.13	75.95	**1.18**						
26/06/2012	5			**0.00**	73.57	67.73	**5.84**						
27/06/2012	6			**0.00**	72.79	69.34	**3.45**						
28/06/2012	7			**0.00**	69.35	65.63	**3.72**						
03/07/2012	8			**0.00**	61.60	54.26	**7.34**						
04/07/2012	9			**0.00**	62.42	53.37	**9.05**						
06/07/2012	10			**0.00**	65.16	57.82	**7.34**						
09/07/2012	11			**0.00**	67.35	47.22	**20.13**						
10/07/2012	12			**0.00**	64.61	49.33	**15.28**						
11/07/2012	13			**0.00**	63.61	47.71	**15.90**						
12/07/2012	14			**0.00**	63.47	48.38	**15.09**						
13/07/2012	15			**0.00**	63.87	45.78	**18.09**						
16/07/2012	16			**0.00**	60.79	54.74	**6.05**						
17/07/2012	17			**0.00**	59.87	54.74	**5.13**						
18/07/2012	18			**0.00**	65.49	50.41	**15.08**						
19/07/2012	19			**0.00**	54.76	49.58	**5.18**						
20/07/2012	20			**0.00**	55.91	41.96	**13.95**						
23/07/2012	21			**0.00**	58.74	53.66	**5.08**						
24/07/2012	22			**0.00**	55.04	49.70	**5.34**						
25/07/2012	23			**0.00**	52.48	47.56	**4.92**						
26/07/2012	24			**0.00**	51.23	44.85	**6.38**						
27/07/2012	25			**0.00**	51.17	43.26	**7.91**						
30/07/2012	26			**0.00**	50.29	45.81	**4.48**						
31/07/2012	27			**0.00**	47.64	44.27	**3.37**						
01/08/2012	28			**0.00**	44.66	40.29	**4.37**						

02/08/2012	29	**0.00**	40.12	33.60	**6.52**						
03/08/2012	30	**0.00**	34.99	30.53	**4.46**						
06/08/2012	31	**0.00**	31.81	25.68	**6.13**	35.24	26.15	**9.09**	106.70	6.27	**100.43**
07/08/2012	32	**0.00**	31.55	25.88	**5.67**	38.92	28.36	**10.56**	108.40	5.01	**103.39**
08/08/2012	33	**0.00**	37.28	32.95	**4.33**	47.03	37.03	**10.00**	136.80	10.20	**126.60**
09/08/2012	34	**0.00**	35.9	30.24	**5.66**	45.73	35.10	**10.63**	149.90	12.18	**137.72**
10/08/2012	35	**0.00**	33.64	27.46	**6.18**	42.26	36.75	**5.51**	170.10	16.92	**153.18**
13/08/2012	36	**0.00**	24.11	18.42	**5.69**	31.79	23.90	**7.89**	182.50	17.89	**164.61**

Annex III: Phases MLSS

Phase I

		Initial (g)	After 105 C (g)	After 550 C (g)	Sample (ml)	S.S (g/l)	Volatile (g/l)
23/05/2011	LINE A	115.2450	115.3805	115.2953	50	**2.71**	**1.01**
	LINE B	114.7657	114.8936	114.8185	50	**2.56**	**1.06**
30/05/2011	LINE A	115.2427	115.4139	115.3141	50	**3.42**	**1.43**
	LINE B	114.7705	114.9264	114.8302	50	**3.12**	**1.19**
06/06/2011	LINE A	78.4039	78.4979	78.4461	50	**1.88**	**0.84**
	LINE B	62.7011	62.7953	62.7333	50	**1.88**	**0.64**
13/06/2011	LINE A	93.6996	93.8315	93.7536	50	**2.64**	**1.08**
	LINE B	109.0088	109.093	109.0403	50	**1.68**	**0.63**
20/06/2011	LINE A	89.7151	89.8535	89.7494	50	**2.77**	**0.69**
	LINE B	114.7753	114.9048	114.8104	50	**2.59**	**0.70**
27/06/2011	LINE A	112.6648	112.8589	112.7148	50	**3.88**	**1.00**
	LINE B	123.8236	123.9952	123.8783	50	**3.43**	**1.09**
30/06/2011	LINE A	112.8622	113.4170	112.9356	50	**11.10**	**1.47**
Anaerobic	LINE B	90.6748	91.1674	90.734	50	**9.85**	**1.18**
30/06/2011	LINE A	108.4204	108.7319	108.4884	50	**6.23**	**1.36**
Anoxic	LINE B	93.7018	93.8410	93.7476	50	**2.78**	**0.92**
30/06/2011	LINE A	74.8492	77.1650	75.0987	50	**46.32**	**4.99**
MLSS last chamber anaerobic line A							

Phase II:

Measure has not been done systematically and therefore is not reported in this annex.

Phase III

		Initial (g)	After 105 C (g)	After 550 C (g)	Sample (ml)	S.S (g/l)	Volatile (g/l)
03/10/2011	AN (A)	108.9015	108.9737	108.9015	50	**1.44**	**0.00**
(Initial)	AN (B)	108.3972	108.5074	108.3972	50	**2.20**	**0.00**
	AX (A)	123.6864	123.7832	123.6864	50	**1.94**	**0.00**
	AX (B)	90.6089	90.7079	90.6089	50	**1.98**	**0.00**
	AE (A)	116.1201	116.2017	116.1201	50	**1.63**	**0.00**
	AE (B)	93.6840	93.7719	93.6840	50	**1.76**	**0.00**
21/10/2011	AN (A)	90.3471	90.7007	90.4935	50	**7.07**	**2.93**
	AN (B)	118.9725	119.2819	119.1804	50	**6.19**	**4.16**
	AX (A)	81.4088	81.5492	81.4905	50	**2.81**	**1.63**
	AX (B)	112.8372	112.9185	112.8888	50	**1.63**	**1.03**
	AE (A)	119.8383	119.9338	119.9025	50	**1.91**	**1.28**
	AE (B)	115.4098	115.5003	115.4613	50	**1.81**	**1.03**
24/10/2011	AN (A)	112.5043	112.7582	112.6295	50	**5.08**	**2.50**
31/10/2011	AN (A)	115.4098	115.7801	115.5725	50	**7.41**	**3.25**
	AN (B)	118.9712	119.3585	119.2295	50	**7.75**	**5.17**
	AX (A)	90.6070	90.7849	90.7178	50	**3.56**	**2.22**
	AX (B)	119.8376	119.9215	119.8864	50	**1.68**	**0.98**
	AE (A)	105.1111	105.3141	105.2607	50	**4.06**	**2.99**
	AE (B)	112.6458	112.7447	112.7022	50	**1.98**	**1.13**
07/11/2011	AN (A)	90.6068	90.9754	90.7542	50	**7.37**	**2.95**
	AN (B)	115.4162	115.9824	115.8256	50	**11.32**	**8.19**
	AX (A)	119.8460	120.0160	119.9386	50	**3.40**	**1.85**
	AX (B)	118.9723	119.0667	119.0236	50	**1.89**	**1.03**
	AE (A)	112.6531	112.9139	112.8221	50	**5.22**	**3.38**
	AE (B)	114.7136	114.8143	114.7698	50	**2.01**	**1.12**

14/11/2011 Before withdr	AN (A)	123.6760	124.1996	123.8684	50	**10.47**	**3.85**
	AN (B)	90.2369	90.8241	90.6142	50	**11.74**	**7.55**
	AX (A)	112.5045	112.6750	112.6021	50	**3.41**	**1.95**
	AX (B)	108.3978	108.4878	108.4421	50	**1.80**	**0.89**
	AE (A)	109.5795	109.8661	109.7515	50	**5.73**	**3.44**
	AE (B)	90.3498	90.4595	90.4118	50	**2.19**	**1.24**
14/11/2011 After withdr	AN (A)	90.6058	90.8736	90.7022	50	**5.36**	**1.93**
	AN (B)	119.8415	119.9581	119.9083	50	**2.33**	**1.34**
Milk reduction after 15/11/11							
21/11/2011 Start Milk (B)	AN (A)	88.1893	88.7012	88.4153	50	**10.24**	**4.52**
	AN (B)	113.9029	114.2454	114.0935	50	**6.85**	**3.81**
	AX (A)	93.6828	93.8302	93.7641	50	**2.95**	**1.63**
	AX (B)	89.4587	89.5582	89.5105	50	**1.99**	**1.04**
	AE (A)	89.4481	89.8314	89.6958	50	**7.67**	**4.95**
	AE (B)	114.7163	114.8275	114.7720	50	**2.22**	**1.11**
28/11/2011	AN (A)	123.6746	124.4059	124.0035	50	**14.63**	**6.58**
	AN (B)	112.5013	112.8900	112.7043	50	**7.77**	**4.06**
	AX (A)	105.1035	105.3283	105.2308	50	**4.50**	**2.55**
	AX (B)	88.1715	88.2710	88.2215	50	**1.99**	**1.00**
	AE (A)	115.2384	115.6780	115.5349	50	**8.79**	**5.93**
	AE (B)	93.6715	93.8090	93.7317	50	**2.75**	**1.20**
29/12/2011	AX (A)	90.6057	90.7009	90.6681	50	**1.90**	**1.25**
	AE (A)	112.6399	112.8423	112.7748	50	**4.05**	**2.70**

Annex IV: Continuous flow test MLSS

		Initial (g)	After 105 C (g)	After 550 C (g)	Sample (ml)	S.S (g/l)	Volatile (g/l)	NOTES
14/106/2012	AN (A)	88.1694	88.2884	88.2098	50	**2.38**	**0.81**	
	AE (A)	118.9507	119.0382	118.9844	50	**1.75**	**0.67**	
18/06/2012	AN (A)	89.4441	89.7143	89.5057	50	**5.40**	**1.23**	
	AX (A)	112.6412	112.7385	112.6822	50	**1.95**	**0.82**	
	AE (A)	113.8641	113.9468	113.9003	50	**1.65**	**0.72**	
27/06/2012	AN (A)	78.3862	78.7855	78.4522	50	**7.99**	**1.32**	
	AX (A)	66.1302	66.2410	66.1644	50	**2.22**	**0.68**	
	AE (A)	70.7443	70.8314	70.7731	50	**1.74**	**0.58**	
02/07/2012	AN (A)	62.6899	63.0514	62.7521	50	**7.23**	**1.24**	
	AX (A)	72.5744	72.6874	72.6008	50	**2.26**	**0.53**	
	AE (A)	77.3464	77.4832	77.3837	50	**2.74**	**0.75**	
09/07/2012	AN (A)	78.3873	78.9392	78.5006	50	**11.04**	**2.27**	
	AX (A)	66.1325	66.3102	66.2052	50	**3.55**	**1.45**	
	AE (A)	62.6897	62.8699	62.7656	50	**3.60**	**1.52**	
16/07/2012								
Before	AN (A)	70.7479	71.4173	70.8625	50	**13.39**	**2.29**	**MLSS Removal**
After	AN (A)	64.5885	64.8725	64.6530	50	**5.68**	**1.29**	
23/07/2012	AN (A)	72.5743	73.0121	72.6542	50	**8.76**	**1.60**	
	AX (A)	88.3634	88.6019	88.4579	50	**4.77**	**1.89**	
	AE (A)	78.3865	78.6423	78.4899	50	**5.12**	**2.07**	
31/07/2012	AN (A)	64.5902	65.2884	64.7758	50	**13.96**	**3.71**	**From 26/07, milk CC= 0.5%**
	AX (A)	61.8794	62.1690	61.9933	50	**5.79**	**2.28**	
	AE (A)	70.7886	71.0108	70.8918	50	**4.44**	**2.06**	

01/08/2012	AN (A)	70.9164	71.1254	70.9993	50	**4.18**	**1.66**	**MLSS Removal**

06/08/2012	AN (A)	61.8823	62.2330	61.9888	50	**7.01**	**2.13**	
	AX (A)	70.9168	71.1828	71.0334	50	**5.32**	**2.33**	
	AE (A)	64.5946	64.8743	64.7299	50	**5.59**	**2.71**	
08/08/2012	AX (A)	72.5807	72.7324	72.6578	50	**3.03**	**1.54**	**MLSS Removal**
	AE (A)	64.1351	64.2684	64.2124	50	**2.67**	**1.55**	
13/08/2012	AN (A)	64.1376	64.7070	64.2866	50	**11.39**	**2.98**	
	AX (A)	72.5798	72.8009	72.6844	50	**4.42**	**2.09**	
	AE (A)	61.8878	62.0658	61.9893	50	**3.56**	**2.03**	

Annex V: Continuous flow test MLSS

1 Easily degradable carbohydrate (chfd)

C-chfd can be represented as glucose ($C_6H_{12}O_6$) (Haug, 1993) according to Jonsson *et al.,* (2005)

$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$

180 g/mol + 192 g/mol for total degradation of chfd give 1.067g (192/180) of COD for each g of chfd.

The COD demand for each g of C (*40% in total) in chfd is 2.62 g (1.067/0.4) (Jonsson *et al.,* 2005)

*** C in total weight: 6x12 + 12x1 + 6x16 = 180; C: 6 x 12 = 72; C: 72/180 = 0.4 (40%)**

2 Medium degradable carbohydrates (chmd)

C-chmd can be represented by polysaccharides ($C_6H_{10}O_5$) as average chemical composition (Haug, 1993) according to (Jonsson *et al.,* 2005).

$3\ C_6H_{10}O_5 + 18\ O_2 \rightarrow 18\ CO_2 + 15\ H_2O$

480 g/mol + 576 g/mol for total degradation of chmd give 1.185g (576/486) of COD for each g of chmd.

The COD demand for each g of C (44.4% in total) in chmd is 2.67g (1.185/0.444) (Jonsson *et al.,* 2005).

3 Slowly degradable organics (chsd)

C-chsd can be represented by the molecule of lignin ($C_{46}H_{38}O_{16}$) according to Sonesson & Jonsson (1996) (Jonsson *et al.,* 2005).

$C_{46}H_{38}O_{16} + 47.5\ O_2 \rightarrow 46\ CO_2 + 19\ H_2O$

846 g/mol + 1520 g/mol for total degradation of chsd give 1.797g (1520/846) of COD for each g of chsd.

The COD demand for each g of C (65.3% in total) in chsd is 2.75g (1.797/0653) (Jonsson *et al.,* 2005).

4 Proteins

A general chemical composition for protein is $C_5H_7NO_2$ proposed by Christensen *et al.,* (2003) according to Jonsson *et al.,* (2005).

$C_5H_7NO_2 + 5\ O_2 \rightarrow 5\ CO_2 + 2\ H_2O + NH_3$

113 g/mol + 160 g/mol for total degradation of protein give 1.42g (160/113) of COD for each g of protein.

The COD demand for each g of C (53.1% in total) in protein is 2.67g (1.42/0.531).

5 Fat and oil

$C_{57}H_{104}O_6$ as general composition of fat and oil is proposed by Christensen *et al.,* (2003) (Jonsson *et al.,* 2005).

$C_{57}H_{104}O_6 + 80\ O_2 \rightarrow 57\ CO_2 + 52\ H_2O$

884 g/mol + 2560 g/mol for total degradation of fat and oil give 2.90g (2560/884) of COD for each g of fat and oil.

The COD demand for each g of C (77.4% in total) in fat and oil is 3.74 g (2.90/0.774).

Table 36: Nutrition facts for long life skimmed milk (UHT)

Typical values	100 ml contains
Proteins	3.1 g
Carbohydrates	4.6 g
Sugar	4.6 g
Fat	1.6 g
Saturated	1.0 g
Salt	0.13 g
Calcium	122 mg

Annex VI NTU Figures of Phases

Figure 99: Phase I Part I and II NTU

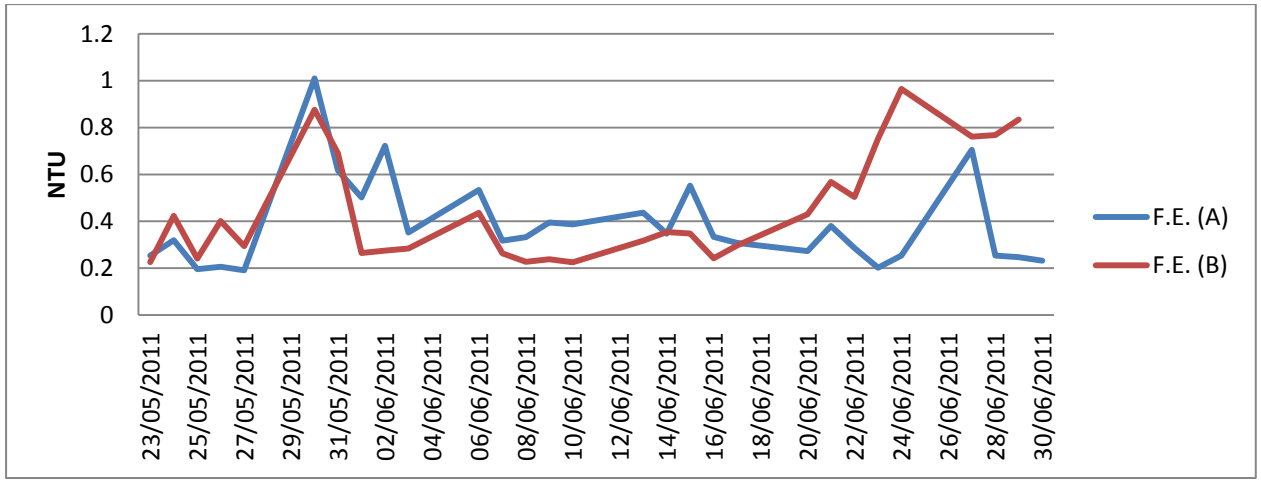


Figure 100: Phase II NTU

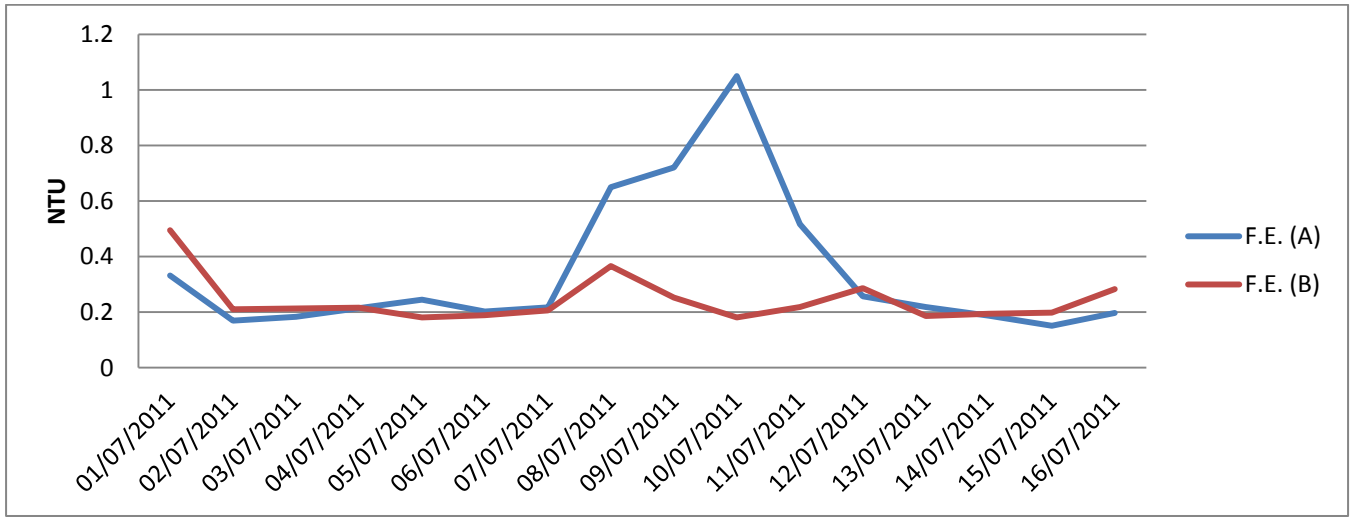


Figure 101: Phase III NTU

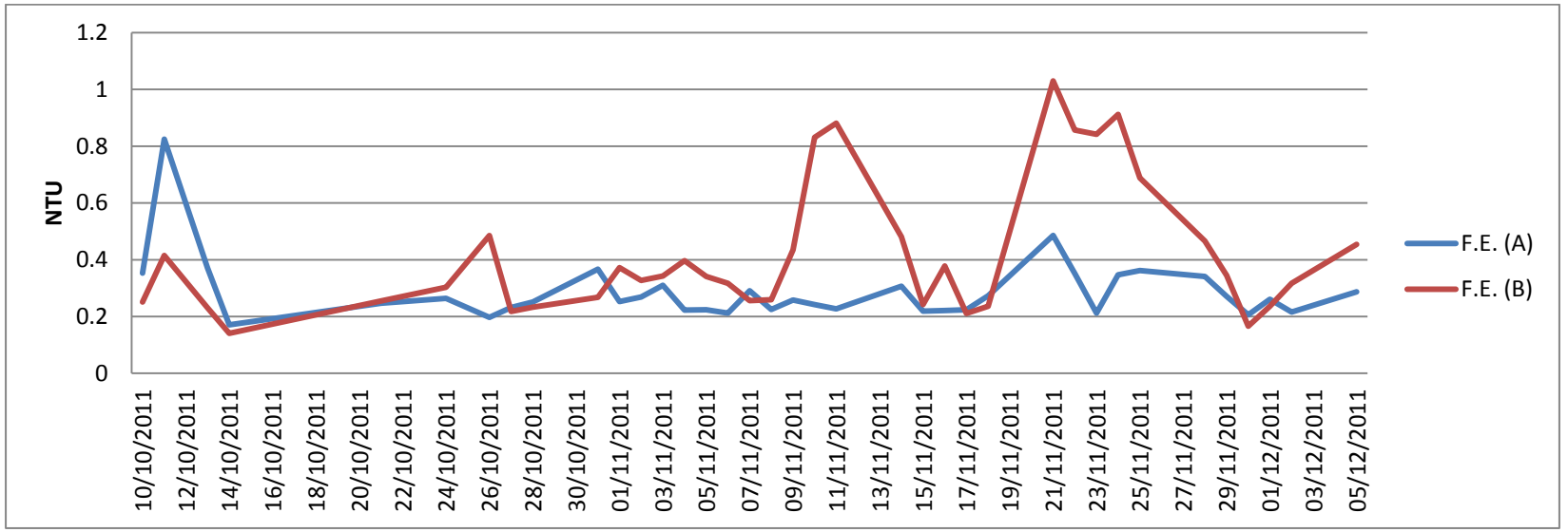


Figure 102: Continuous flow NTU