TAMPERE UNIVERSITY OF TECHNOLOGY

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PRODUCTION AND UTILIZATION OF MUNICIPAL WASTEWATER SLUDGES

Master of Science Thesis

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ABSTRACT

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Present wastewater treatment processes, among which the activated sludge process is the most widely used, produce massive volumes of excess sludge that requires proper treatment. Excess sludge typically consists of feasible organic substances such as enzymes and proteins, and can offer many beneficial utilization possibilities. Thus, it is reasonable that in addition to being perceived as bare waste, new techniques and methods for sludge utilization and disposal are being constantly studied. Recent concerns on the climate change and depletion of mineral oil have awakened interest on recovering the lipids from the excess sludge. Techniques for converting the sludge into biodiesel exist, but the current technology is not economically feasible. Thus, novel and more cost effective techniques are being developed. One possibility is to combine biological wastewater treatment and enhanced production of lipid rich sludge.

The main goal of this thesis was to study if microbial lipid accumulation in municipal wastewater treatment plant's sludge could be enhanced by manipulating the carbon to nitrogen ratio (C/N ratio). Experiments were conducted using batch bottle cultivations, and the C/N ratio was manipulated by adding glucose. With initial glucose addition, the lipid content (mass-% of dried sludge) and concentration (mg lipids/L cultivation) increased from initial in three out of four batch bottle experiments. The highest lipid content (9.2 mass-% compared to initial 6.3 mass-%) was achieved with initial glucose addition of 4 g/L. The highest lipid concentration (570 mg/L compared to initial 516 mg/L) was achieved with initial glucose addition of 10 g/L. In addition, over 100 mg/L increases in lipid concentration were detected with initial glucose addition of 10 g/L in both activated sludge and excess sludge experiments.

In addition to batch bottle experiments, a laboratory scale activated sludge reactor was constructed mainly for gathering useful insight into future experiments. Sludge and lipid production was studied using municipal wastewater as an influent. In order to simulate different organic loadings, glucose was added to the influent wastewater. With higher organic loadings, slightly higher lipid content (mass-% of dried sludge) was achieved compared to initial. Sludge's lipid concentration (mg/L) was lower because of the decreased concentration of suspended solids in the laboratory scale reactor.

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Nykyiset jätevedenpuhdistusmenetelmät, joista yleisin on aktiivilieteprosessi, tuottavat valtaisat määrät ylijäämälietettä. Muun muassa taudinaiheuttajien ja raskasmetallien takia sekä lietteen käsittely että loppusijoitus on suoritettava huolellisesti. Haitallisten komponenttien lisäksi jätevesiliete sisältää myös hyödynnettävissä olevia orgaanisia fraktioita, kuten lipidejä, entsyymejä ja proteiineja, joiden hyödyntämiseen tähtääviä tekniikoita tutkitaan enenevässä määrin. Huoli fossiilisten polttoaineiden käytön vaikutuksesta ilmastonmuutoksen etenemiseen ja mineraalisten öljyvarantojen ehtymisestä on herättänyt kiinnostuksen jätevesilietteen lipidivarantojen hyödyntämisestä biodieselin tuotantoon. Tämänhetkisin menetelmin biodieselin valmistaminen jätevesilietteestä on mahdollista, mutta taloudellisesti kannattamatonta. Tämän vuoksi on syytä kehittää uusia, halvemmat tuotantokustannukset mahdollistavia tekniikoita. Yksi esille tulleista vaihtoehdoista on biologisen jätevedenpuhdistuksen sekä lipidirikkaan lietteen tuotannon yhdistäminen.

Tämän diplomityön päätarkoitus oli tutkia mikrobien lipidituotannon tehostamista aktiivilietepuhdistamon lietteessä manipuloimalla lietteen hiili/typpi-suhdetta. Kokeet suoritettiin panospullokasvatuksina laboratorio-olosuhteissa, ja hiili/typpi-suhdetta muunneltiin lisäämällä kasvatuksiin glukoosia. Kolmessa neljästä suoritetusta kokeesta saavutettiin glukoosilisäyksen avulla hienoista kasvua sekä lietteen lipidipitoisuudessa (massa- % lietteen kuivapainosta) että lipidikonsentraatiossa (mg lipidejä/l kasvatusta). Korkein lipidipitoisuus (9,2 massa- %, vrt. alkuperäinen 6,3 massa- %) saavutettiin, kun kasvatuksen alussa glukoosikonsentraatio oli 4 g/l. Korkein lipidikonsentraatio (570 mg/l, vrt. alkuperäinen 516 mg/l) saavutettiin, kun kasvatuksen alussa glukoosikonsentraatio oli 10 g/l. Yli 100 mg/l lipidikonsentraation kasvu havaittiin kahdessa kokeessa.

Pullokokeiden lisäksi kokeita tehtiin laboratoriomittakaavan aktiivilietereaktorilla. Lietteen ja lipidien tuottoa tutkittiin käyttämällä syötteenä kunnallista jätevettä (Viinikanlahden jätevedenpuhdistamo, Tampere). Puhdistustulosten osalta toinen kahdesta reaktoriajosta oli verrattain onnistunut, ja paljon hyödyllistä tietoa reaktorin toiminnasta kerättiin tulevia kokeita varten. Korkeamman orgaanisen kuormituksen simuloimiseksi jäteveteen lisättiin glukoosia. Kuormitusta kasvattamalla saavutettiin hieman kasvua lietteen lipidipitoisuudessa. Liian tehokas lietteenpoisto kuitenkin laski lietekonsentraatiota ja täten myös lipidikonsentraatiota laboratorioreaktorissa.

PREFACE

This study was conducted at Tampere University of Technology at the Department of Chemistry and Bioengineering. I am grateful to the supervisors and examiners of this thesis, Professor Jaakko Puhakka, Researcher Aino-Maija Lakaniemi and D.Sc. (Tech) Perttu Koskinen for their guidance and advice. I want to thank Neste Oil Corporation for funding the study. I also want to thank co-thesis worker Jatta Marjakangas, Chief Laboratory Technician Tarja Ylijoki-Kaiste and all the other personnel at the Department of Chemistry and Bioengineering for all their help, and for creating a cheerful working environment. Also personnel at Viinikanlahti wastewater treatment plant, especially Heikki Sandelin, deserve thanks for their cooperation.

Finally, I want to express my greatest gratitude to my parents for all the love and support.

Lieto, September 2011

Petteri Laaksonen

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ABBREVIATIONS AND NOTATION

BOD_5	5-day Biological Oxygen Demand
BOD_{7ATU}	7-day Biological Oxygen Demand, (+Allylthiourea)
C/N ratio	Proportion of Carbon to Nitrogen
COD_{tot}	Chemical Oxygen Demand of Total Content
COD_s	Chemical Oxygen Demand of Soluble Content
DO	Dissolved Oxygen Concentration
DOC	Dissolved Organic Carbon
FAME	Fatty Acid Methyl Ester
F/M ratio	Food to Microorganism Ratio
EPS	Extracellular Polymeric Substances
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
NH_4-N	Ammonium Nitrogen
N_{tot}	Total Nitrogen
PAHs	Polycyclic Aromatic Hydrocarbons
PAO	Polyphosphate Accumulating Organism
SRT	Solids Retention Time
TAG	Triacylglyceride
TOC	Total Organic Carbon
TS	Total dry Solids
TSS	Total Suspended Solids
VOCs	Volatile Organic Compounds
VSS	Volatile Suspended Solids
WWTP	Wastewater Treatment Plant

Domestic Wastewater	Wastewater from domestic households
Municipal Wastewater	Wastewater arriving in the municipal wastewater treatment plant. In addition to domestic wastewater, may contain industrial wastewaters, infiltration/inflow water and storm water.
Plug Flow Reactor	A technical system, in which the flow is considered to travel as stationary segments having similar volumes, “plugs”.

1 INTRODUCTION

The management of human waste, or lack of waste treatment, will always be a challenge facing the world's populations. More and more people live in densely populated urban areas where effective waste management is crucial for health and for the environment. Although there are other options, e.g. a dry toilet, water is still widely used for carrying excrements as well as many other wastes away from their sources. Especially in areas with poor fresh water resources, the circulation and reuse of wastewater are becoming more widely used while the techniques are improving. These circumstances have lead into emergence of massive wastewater streams. (LeBlanc et al. 2008; Langenbach et al. 2009.)

Present wastewater treatment processes, among which the activated sludge process is the most widely used, produce massive volumes of pathogenic excess sludge and other organic material, a.k.a. biosolids, which require proper treatment. The treatment of excess sludge may constitute approximately one third of the wastewater treatment plants operating costs, although in addition to being bare waste, excess sludge can offer many beneficial utilization possibilities. It can serve as a valuable source of nutrients for agriculture and as a source of energy usually via biogas production or combustion. In addition, excess sludge typically consists of feasible organic substances such as enzymes and proteins. Thus, it is reasonable that new techniques and methods for sludge utilization and disposal are being constantly studied. (Rittmann & McCarty 2001; LeBlanc et al. 2008; Muga & Mihelcic 2008; Hwang et al. 2008; Rantanen et al. 2008; Santala & Etelämäki 2009; Finland's Environmental Administration 2011.)

Recent concerns on the climate change and depletion of mineral oil have awakened interest on recovering the lipids from the excess sludge. Techniques for converting the sludge into biodiesel are already available, but costs are too high for economically beneficial production. Thus, novel and more cost effective techniques are being developed. One possibility is to combine biological wastewater treatment and enhanced production of lipid rich sludge. (Angerbauer et al. 2008; Mondala et al. 2009; Revellame et al. 2009; Pokoo-Aikins et al. 2010; Mondala et al. 2011.)

The main goal of this thesis was to study if microbial lipid accumulation in excess sludge could be enhanced by manipulating the carbon to nitrogen ratio (C/N ratio). Experiments were conducted using batch bottle cultivation, and the C/N ratio was manipulated by adding glucose. In addition to batch bottle experiments, two experiments were conducted with a laboratory scale activated sludge reactor. With the reactor, sludge and lipid productions were studied using municipal wastewater as an influent. In order to simulate different organic loadings, glucose was added to the influent wastewater.

2 MUNICIPAL WASTEWATER

Every community produces both solid and liquid wastes. Municipal wastewater may be defined as a combination of the liquid and solid, water-carried wastes removed from residences and municipal, commercial as well as industrial establishments. Also ground-, surface-, and storm waters are often carried off into same pipelines with other wastewater. Untreated wastewater contains plenty of organic material that is biologically degradable and therefore causes for example malodours and excessive oxygen consumption in receiving waters. In addition, untreated wastewater contains numerous pathogenic micro-organisms from human intestinal tract or industrial wastes. Wastewater also contains nutrients such as nitrogen and phosphorus that stimulate the growth of aquatic organisms leading to eutrophication. An increasing problem is the existence of toxic, mutagenic and carcinogenic compounds as well as endocrine disrupting chemicals in wastewater. Those compounds are detrimental to both animals and plants, and they cause problems in biological wastewater treatment. For these reasons, the immediate and fluid removal of wastewater from its sources of generation, followed by efficient treatment, reduction or removal of unwanted constituents, and reuse or dispersal into the environment is necessary for public health and the environment. It is also important to relevantly treat the solid wastes carried by wastewaters, as well as wastes from biological and chemical wastewater treatment processes. (Metcalf & Eddy, Inc., 1991; Metcalf & Eddy, Inc., 2003; Karttunen et al. 2004, Seviour & Nielsen 2010)

For proper wastewater treatment, the knowledge of the characteristics of wastewater in question is necessary. Chapter 2 presents the fundamental constituents usually found in municipal wastewater. The role of municipal wastewater constituents and quality in activated sludge wastewater treatment will be further discussed in Chapter 3.

2.1 Organic Constituents of Wastewater

According to Metcalf & Eddy, Inc. (2003), in a medium strength wastewater, total suspended solids (TSS) concentration is near 720 mg/L. Approximately 75% of the TSS is organic. The organic matter of municipal wastewater is typically composed of proteins (40 - 60%), carbohydrates (25 - 50%) and lipids (8 - 12%). Another important organic wastewater compound is urea, although it degrades so rapidly that it is usually found only in the very fresh wastewater. There are also diverging research results on domestic wastewater composition, for example Huang et al. (2010) reported that fibers, proteins and sugars accounted for approximately 21, 12 and 11% of total organic carbon (TOC) in the wastewater, respectively. The final composition of wastewater arriving in the

wastewater treatment plant (WWTP) is depending on various parameters, such as the proportion of industrial wastewaters and drainage water. Another variable is the composition of organic waste carried by water, such as food products etc. that are put into the sewer. This waste stream differs between each country and even between regions, depending on how the treatment of organic, food-based waste is arranged. (Metcalf & Eddy, Inc. 1991; Metcalf & Eddy, Inc. 2003; Karttunen et al. 2004)

Organic compounds are normally composed of carbon, hydrogen and oxygen, and especially in proteins also nitrogen, sulphur, phosphorus and iron are present. In addition to proteins, carbohydrates, lipids and urea, municipal wastewater typically contains a large number of different synthetic organic molecules such as pesticides, agricultural chemicals and pharmaceutical compounds. These molecules are usually present in small quantities, with structures ranging from simple to extremely complex. (Metcalf & Eddy, Inc. 2003; Karttunen et al. 2004)

2.1.1 Proteins

Proteins are the principal constituents of the animal organism and essential for structure, function and reproduction of all living matter. All raw animal and plant food products contain proteins, although in plants proteins occur to a lesser extent than animals. Proteins are naturally occurring polymers composed of a large number of amino acid units joined together by amide or peptide bonds. Chemical structure of proteins is complex and unstable, which exposes proteins to various forms of decomposition. There are proteins both soluble and insoluble in water. The molecular weights of proteins are relatively high, ranging from 20 000 to 20 million. (Metcalf & Eddy, Inc. 1991; Hart et al. 2003.)

In addition to carbon, hydrogen and oxygen, proteins contain as their distinguishing characteristic about 16 percent of nitrogen. Urea and proteins are the main sources of nitrogen in the domestic wastewater. (Metcalf & Eddy, Inc. 1991)

2.1.2 Carbohydrates

Carbohydrates are polyhydroxyaldehydes, polyhydroxyketones, or substances that give such compounds via hydrolysis. Carbohydrates are usually classified as monosaccharides, oligosaccharides or polysaccharides, according to their structure. (Hart et al. 2003.) According to Metcalf & Eddy, Inc. (1991) carbohydrate group includes sugars, starches, cellulose and wood fibre, which all are found in wastewater.

Molecular formulas of carbohydrates can be expressed as hydrates of carbon. The common carbohydrates contain five, six or more carbon atoms and oxygen and hydrogen atoms in the same proportions than in water. Some carbohydrates, such as sugars, are soluble in water while others such as starches are insoluble. The starches are more stable than sugars, but can be converted into sugars by microbial activity or by dilute mineral acids. (Metcalf & Eddy, Inc. 1991)

2.1.3 Lipids

The term lipids can be characterized as fats, oils, greases, waxes, fatty acids and other related constituents such as cholesterols, some vitamins and hormones. Lipids are one of the most important compounds of natural foodstuff and many synthetic compounds and emulsions. They are constituents of plants or animals, and characterized by their solubility properties. Lipids are insoluble in water, but they can be extracted from plant or animal matter by non-polar organic solvents such as chloroform, diethyl ether, hexane or toluene. Lipids may vary significantly in chemical structure, even though they have similar solubility properties. Some lipids are esters, some are hydrocarbons, some are acyclic and others are cyclic, even polycyclic. Other lipid-like compounds include mineral oils such as kerosene, lubricating oils and road oils. According to Chipasa & Medrzycka (2006), lipids bring on approximately 30-40% of the municipal wastewaters total chemical oxygen demand. (Metcalf & Eddy, Inc. 1991; Quéméneur & Marty 1994; Raunkjær et al. 1994; Hart et al. 2003; Chipasa & Medrzycka 2006; Manahan 2010)

The most common lipids are fats and oils. Although oils are liquids when fats are solids, they have quite similar basic organic structures. Fats and oils are triacylglycerides (TAGs), a.k.a. fatty acid triesters of glycerol. The triacylglycerides are formed from the glycerol and three long chain fatty acids (such as stearic acid, but the chains may also differ from each other), as shown in Figure 2.1. Sources of animal based fats and oils are butter, margarine, lard and the fatty proportions of meat. Plant lipids are often oils and origin from seeds, such as rapeseed and cottonseed, beans, nuts and certain fruits. (Metcalf & Eddy, Inc. 1991; Hart et al. 2003)

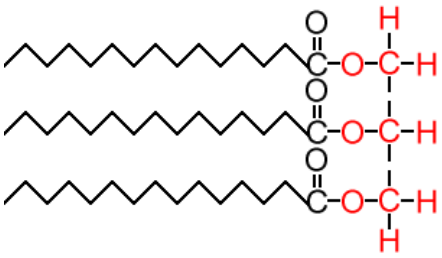

Figure 2.1. *General formula of a triacylglyceride. The glycerol molecule is marked by red colour, and the three fatty acid chains are black. (Modified from Manahan 2010)*

An important lipid class consists of phospholipids, which constitute about 40% of cell membranes, the remaining 60% being proteins. Phospholipids can be regarded as triacylglycerides in which one ester group is replaced by an orthophosphoric acid, as in Figure 2.2. Phospholipids are essential constituents of cell membranes. In the membranes, phospholipids arrange themselves in bilayers, with the two hydrophobic hydrocarbon “tails” pointing inside and the hydrophilic phosphate ends pointing outside of the membrane. Cell membranes have a key role in biology, controlling diffusion of substances in and out of the cells. (Hart et al. 2003; Manahan 2010)

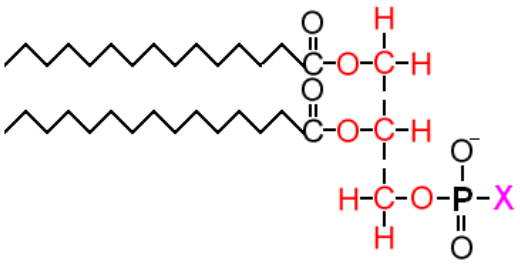

Figure 2.2. *General formula of a phospholipid. X can be a number of different substituents, such as choline or ethanolamine. (Modified from Hart et al. 2003)*

Waxes are produced by both plants and animals, mainly as protective coatings. Waxes end up in the municipal wastewater through a number of common products such as cosmetics, polishes, ointments and other pharmaceutical products. Waxes differ from fats and oils in that they are simple monoesters. Both the acid and alcohol portions of a wax molecule have long saturated carbon chains. A common wax used in cosmetics and pharmaceuticals is cetyl palmitate, which is extracted from the sperm whales blubber. (Hart et al. 2003; Manahan 2010)

As discussed later in Chapter 3, lipids can cause various problems in wastewater treatment plants, including sludge flotation and promotion of growth of filamentous microorganisms, which cause bulking and foaming. In the sewers, lipids, especially mineral oils, tend to coat surfaces and cause maintenance problems. If lipids end up in the receiving waters, they may interfere with the living organisms and cause unsightly floating matter and films. (Metcalf & Eddy, Inc. 1991; Quéméneur & Marty 1994; Raunkjær et al. 1994; Chipasa & Medrzycka 2006; Chipasa & Medrzycka 2008)

Recent concerns on the climate change and depletion of mineral oil have awakened interest on recovering the lipids from municipal wastewater treatment, especially from the excess sludge that is produced at biological WWTPs, and use them as a feedstock for biodiesel. Techniques for converting excess sludge into biodiesel are already available, but the costs are too high for economically beneficial production. Thus, novel and more cost effective techniques are being developed. One possibility is to combine biological wastewater treatment and enhanced production of lipid rich sludge for biodiesel feedstock. Municipal wastewater sludges and lipid utilization are more extensively discussed in Chapter 4. (Angerbauer et al. 2008; Mondala et al. 2009; Revellame et al. 2009; Pokoo-Aikins et al. 2010; Mondala et al. 2011.)

2.1.4 Other Organic Substances

Surfactants, or surface active agents, are large organic molecules that are slightly soluble in water. They contain polar and nonpolar parts and therefore act at the surfaces where different substances meet. Examples of surfactants are soaps and synthetic detergents. With emulsifying power and surface action, soaps and detergents are able to detach for example dirt, grease and oil particles from the surface so that they can be washed away. Surfactants tend to collect at the air-water interface. In wastewater treat-

ment plants, surfactants collect on the surface of the air bubbles during aeration and create very stable foam, causing foaming problems in aeration basins and also in the receiving waters. (Metcalf & Eddy, Inc. 1991; Hart et al. 2003)

Both EU and USA environmental institutions have been identifying and categorising substances as “priority pollutants”. Priority pollutants, both organic and inorganic, are selected on the basis of their known or suspected carcinogenicity, mutagenicity, teratogenicity, or high acute toxicity. Examples of priority pollutants are volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), pesticides and agricultural chemicals, disinfection by-products, heavy metals and halogenated compounds. The concentrations of these chemicals may not be high, and they are not common constituents of domestic wastewater but result primarily from surface runoffs from agricultural, vacant, industrial and waste lands. However, even small concentrations of priority pollutants may cause problems in wastewater treatment processes, or they can result in contamination and harm the flora and fauna of the receiving waters. Within wastewater collection treatment systems, organic priority pollutants may be removed, transformed, passed through the system or even generated. (Metcalf & Eddy, Inc. 1991; Gasperi et al. 2008)

2.1.5 Measurement of the Organic Content

The most commonly used parameter for determining the organic content of wastewater is biological oxygen demand of 5 days incubation of the wastewater sample (BOD_5). In Finland, the established practise is to determine the BOD of 7 days incubation (BOD_7). (Metcalf & Eddy, Inc. 1991; Karttunen & Tuhkanen 2003) BOD test determines the approximate quantity of oxygen that will be required to biologically stabilize the present organic matter. Thus, BOD test can also be used to determine the size of wastewater treatment facilities and in treatment efficiency measurement. Although BOD test is widely used, it has few limitations when measure of total organic content of wastewater is desired – BOD only measures the biodegradable organics, and the measurement period may not correspond to the point when all the soluble organic matter has been used. (Metcalf & Eddy, Inc. 2003)

Aim of the BOD analysis is to estimate the concentration of biologically degradable organic matter in the wastewater. During the hydrolysis of the proteins, noncarbonaceus matter such as ammonia is produced. Nitrifying bacteria, mainly *Nitrosomonas* and *Nitrobacter* genera oxidise ammonia to nitrite and subsequently nitrate, consuming oxygen. When nitrification occurs in the BOD test, the results falsely indicate higher biodegradable organic content than there actually is. In BOD analysis, allylthiourea (ATU) can be added into the samples in order to rule out the oxygen demand caused by nitrification. (SFS 1979; Metcalf & Eddy, Inc. 2003.)

In chemical oxygen demand (COD) analysis, the organic material is chemically oxidized using dichromate or permanganate, and oxygen equivalent is calculated. The permanganate oxidation method is more sensitive than dichromate oxidation, and usually applied with waters containing only low concentrations of organic substances (SFS

3036). With wastewater, the dichromate oxidation method is often used. The COD is usually fractionated into soluble and total (COD_s and COD_{tot}). In principle, sample's COD is higher than BOD. Some reasons for higher COD are difficultly biodegradable organic substances that can be oxidized chemically, as well as possible toxic organic substances that inhibit the microbial growth. Also certain inorganic substances can be oxidized by dichromate, thus leading to higher COD. (Metcalf & Eddy, Inc. 2003)

The total organic carbon (TOC) or dissolved organic carbon (DOC) content of wastewater can be analyzed with special equipment. For example for biological wastewater treatment, the TOC does not give any information on how biodegradable or inhibiting the organic substances are. In some cases, it is possible to relate BOD, COD and TOC of a given wastewater and in these cases TOC measurement is useful because it is quick to conduct. In addition, measurement of volatile suspended solids (VSS) is easy and quick to conduct and gives useful insight for organic material estimations. (Metcalf & Eddy, Inc. 2003; Karttunen et al. 2004.)

2.2 Inorganic Constituents of Wastewater

Inorganic nonmetalic and metallic constituents derive into the wastewater from the background levels of fresh water treatment, domestic and industrial use. Several inorganic constituents of wastewater have a great role in control of the wastewater treatment processes as well as in the quality of wastewater treatment effluent. Especially biological wastewater treatment processes are dependent of proper concentrations of nutrients such as nitrogen and phosphorus, as well as the key trace nutrients such as iron, sulphur, zinc, copper and molybdate. The other side is that especially nitrogen and phosphorus are essential to the growth of protista, plants and algae, and therefore cause eutrophication and oxygen depletion in the receiving waters. (Rittmann & McCarty 2001; Metcalf & Eddy, Inc. 2003; Karttunen et al. 2004.)

In Finland, nitrogen is usually released into municipal wastewater at a rate of 12-15 g/inhabitant/day. Approximately half of the nitrogen in wastewater is bound into organic compounds, mainly proteins and urea. The other half of the nitrogen is present as ammonium ions. (Karttunen et al. 2004) During wastewater treatment process nitrogen can undergo several transformations from organic nitrogen into ammonium, from ammonium into organic nitrogen in bacterial cells, or from ammonium into nitrite, nitrate and finally nitrogen gas through nitrification/denitrification process. Wastewater ammonium is the most detrimental form of nitrogen for receiving waters, because its oxidation consumes alkalinity and oxygen. Ammonium is also toxic to fish. Thus, ammonium needs to be transformed into nitrate or removed via denitrification. (Metcalf & Eddy, Inc. 2003; Karttunen et al. 2004)

Phosphorus is released into municipal wastewater at a rate of 5-9 g/inhabitant/day. Usually phosphorus is present in organic form, orthophosphate or complex phosphate. The organically bound phosphorus is usually of less importance in domestic wastewaters, although it can have a larger role in industrial wastewaters and wastewater

sludge. The concentration of complex phosphates is depending on the concentration of synthetic detergents in wastewater. Complex phosphates undergo hydrolysis in aqueous solutions, and revert to orthophosphate form; however, this hydrolysis is usually quite slow. In Finland, phosphorus is usually precipitated with iron sulphate before biological wastewater treatment process. (Karttunen et al. 2004)

In addition to nutrients, wastewater contains other inorganic constituents such as, chlorides, sulphur, heavy metals, gases and odours. Also hydrogen ion concentration (pH) and hydroxide, carbonate and bicarbonate concentration (alkalinity) can be considered as inorganic constituents. These constituents have their own effects on concentration and state of the other chemical and biological characteristics of the wastewater, as well as they may affect the pipelines and WWTP processes. (Metcalf & Eddy, Inc. 2003.)

2.3 Wastewater Composition

Typical compositions of different municipal wastewaters are presented in Table 2.1. Total wastewater flow typically consists of four flow fractions: domestic wastewater, industrial wastewater, infiltration/inflow and storm water. Thus, differences in each water fraction and discharge volume affect the total wastewater composition. (Metcalf & Eddy, Inc. 2003) The composition of domestic wastewater varies with social customs, water usage rates, culture, general lifestyle and dietary habits. For example, the quantity of organic, food based waste carried by wastewater is different between nations. (Metcalf & Eddy, Inc. 2003, Seviour & Nielsen 2010.)

Industrial wastewater composition is determined by the products and processes of the industrial plant. In Finland, before carrying the wastewaters to the municipal WWTP, the industrial plant needs to make a contract with the WWTP. Municipal WWTPs can also set their own limitations and instructions regarding to industrial wastewater composition and volume. Industrial wastewaters also need to meet the respective legislations. (FIWA 2011)

The volumes of infiltrated waters, storm waters and other drainage waters depend on various parameters such as annual rainfall, evaporation and drainage systems. Especially old sewer systems are usually combined sewers, where drainage waters may cause overflow of the total wastewater and dilute the wastewater carried to the WWTP. Drainage waters may also carry harmful compounds such as fuel additives or heavy metals. (Metcalf & Eddy, Inc. 2003; Karttunen et al. 2004; FIWA 2011)

Table 2.1. *Typical compositions of different municipal wastewaters.*

Constituent	Unit	Weak[1]	Medium[1]	Strong[1]	Finland[2]
Total Solids (TS)	mg/L	350	720	1200	350 - 600
Total Suspended Solids (TSS)	mg/L	100	220	350	150 - 200
Volatile Suspended Solids (VSS)	mg/L	80	165	275	120 - 150
BOD_5	mg/L	110	220	400	125 - 175[3]
COD	mg/L	250	500	1000	300 - 450
TOC	mg/L	80	160	290	n.i.a.
Total Nitrogen (N_{tot})	mg/L	20	40	85	25 - 40
Ammonium Nitrogen (NH_4-N)	mg/L	12	25	50	15 - 25
Total Phosphorus (P_{tot})	mg/L	4	8	15	6 - 8
Potassium (K)	mg/L	n.i.a.	n.i.a.	n.i.a.	10 - 15
Chloride (Cl^-)	mg/L	-	-	-	25 - 75
Grease	mg/L	50	100	150	-
pH		n.i.a.	n.i.a.	n.i.a.	6 - 8

n.i.a. = no information available

[1)] Classification according to Metcalf & Eddy, Inc., (1991)

[2)] Typical concentrations in Finnish municipal wastewater according to Karttunen et al. (2004)

[3)] BOD_{7ATU}

2.4 Biological Characteristics of Wastewater

Organisms found in wastewater include bacteria, fungi, algae, protozoa, plants, animals and viruses. There are two major reasons why information on the biological characteristics of wastewater is important. One reason is the control of deceases caused by pathogenic organisms of human origin. The other reason is the fundamental role played by bacteria and other micro-organisms in the decomposition and stabilization of organic matter. This happens in nature, but the feature can also be exploited in biological wastewater treatment, as discussed in more detail in Chapter 3. (Metcalf & Eddy, Inc. 2003)

In healthy people, the majority of excreted pathogenic organisms are harmless commensal bacteria, but they are present in very large numbers, more than 10^9 cells per gram of faeces. However, people may also be suffering from or carrying certain pathogenic organisms, which are excreted in wastewater. (Seviour & Nielsen 2010) Typically pathogenic organisms of wastewater cause diseases of the gastrointestinal tract, such as diarrhoea and typhoid fever. Numbers of pathogenic organisms present in wastewater are usually few and difficult to identify. Therefore microorganisms which are more numerous and more easily tested, such as total coliform bacteria and enterococci, are commonly used as indicator organisms for the target pathogens. (Metcalf & Eddy, Inc. 2003.)

2.5 Wastewater Treatment

Wastewater can be treated with various methods and their combinations. Wastewater treatment solutions consist of physical unit operations and chemical or biological unit processes. In physical unit operations the change in water quality is achieved using physical phenomenona. Typical physical unit operations in wastewater treatment are screening, mixing, settling and filtration. In chemical unit processes such as precipitation and disinfection, the wastewater contaminants are removed by adding chemicals or utilizing chemical reactions. Biological unit processes utilize biological reactions in order to remove wastewater contaminants such as BOD and nutrients. A typical example on the placing of physical unit operations and chemical and biological unit processes on WWTP is presented in Figure 2.3. (Metcalf & Eddy, Inc. 2003; Karttunen et al. 2004)

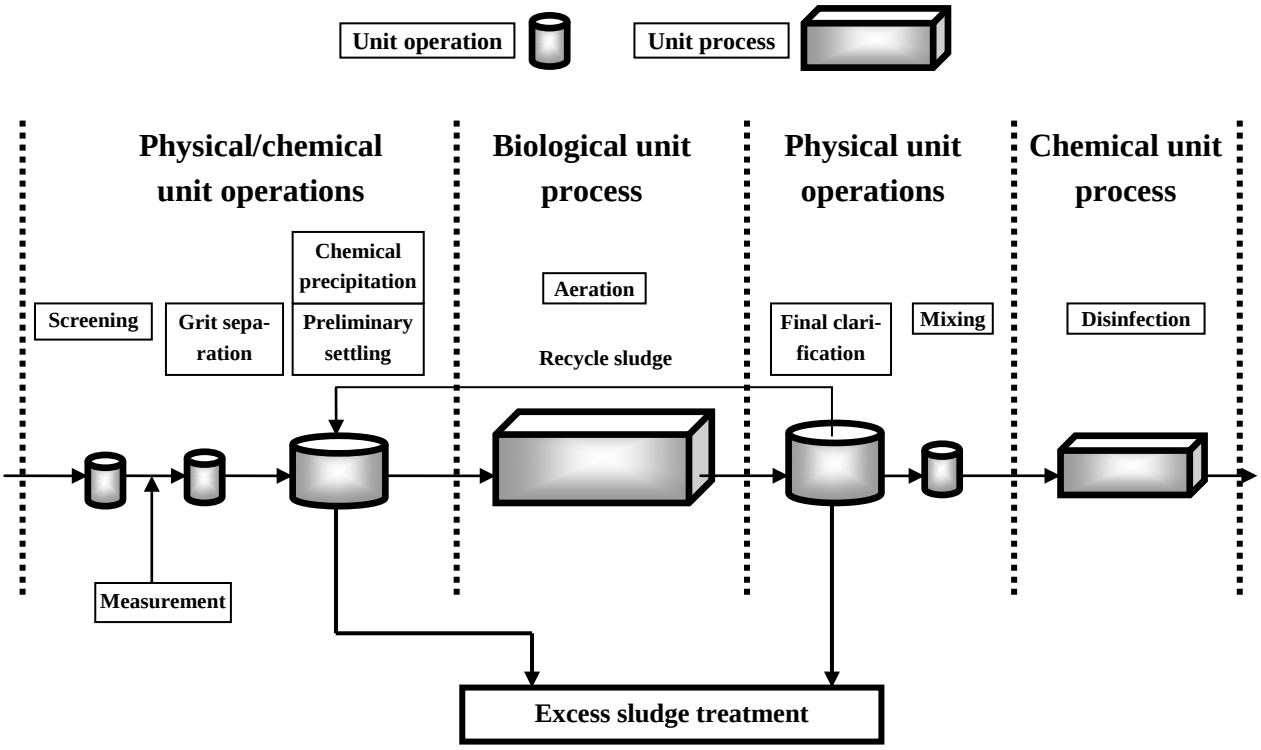

Figure 2.3. *A typical example on the placing of physical unit operations and chemical and biological unit processes on WWTP (modified from Karttunen et al. 2004)*

The advantages of biological unit processes over chemical unit processes include lower energy and chemical consumption as well as lower waste production. Because of those advantages and the extensive research on the subject, biological unit processes have become a large part of conventional wastewater treatment. The most widely used,

most known and most typical biological unit process is the activated sludge process, which nowadays is a fundamental part of almost every large scale WWTP in Finland, although advanced WWTPs typically combine various unit operations and both chemical and biological processes. (Rittmann & McCarty 2001; Metcalf & Eddy, Inc. 2003; Karttunen et al. 2004)

Wastewater treatment effluent is typically carried off to receiving waters. All the other material produced at the WWTP, such as screenings, grit, foam and sludge require certain stage of secondary treatment. The excess sludge forms quantifiable the largest fraction of WWTPs wastes, and treatment and utilization of the sludge is one of the most challenging aspect of wastewater treatment. Because of its essential role in wastewater treatment and sludge production, the activated sludge process is discussed in more detail in the following Chapter 3.

3 ACTIVATED SLUDGE PROCESS

The activated sludge process represents probably the largest biotechnology industry in the world. Activated sludge process is the most widely used and studied biological process in municipal and industrial wastewater treatment. It differs from most conventional biotechnology processes which require pure cultures and controlled aerobic fermentations for large scale production of economically important products. Activated sludge process needs to be robust, and its microbial communities are mixed populations having to deal with various inorganic and organic compounds. The influent compounds also enter the system at irregular concentrations and differ in their chemical compositions and molecular or particle sizes. In addition to faecal material, domestic and industrial wastewaters carry a range of naturally occurring and xenobiotic organic compounds. Many of these compounds will be adsorbed and/or degraded by the bacterial populations that are enriched in the activated sludge process. The ecology of the process can change daily, leading to significant problems such as sludge bulking or poor treatment efficiency. (Rittmann & McCarty 2001; Metcalf & Eddy, Inc. 2003; Seviour & Nielsen 2010)

Activated sludge process was discovered in England in 1914, when E. Ardern and W.T. Lockett detected that aeration of sewage led to formation of flocculent suspended particles, flocs. They also discovered that the time to remove organic contaminants was reduced when the flocs were held in the system. The resulting sludge from settling was referred to be “activated”, and so was activated sludge process born. The process came into common use in early 1920s, and in the beginning the most commonly used activated sludge systems had strong plug flow reactor characteristics. The early development of activated sludge process included the debate over whether the obtained removal was due to physical or biological phenomena. By 1930, the evidence in favour of biological process was sufficiently convincing. Finally, by the 1950s and 1960s, a theory of operation had developed and rational designs based on the characteristics of the wastewater could be achieved. In the late 1960s the discharge of industrial wastewaters into domestic wastewater collection systems increased. The use of conventional plug flow reactors became problematic because of the toxic effects of industrial wastes. Other activated sludge process modifications were developed to treat larger volumes, allowing greater dilution and thus mitigating the toxic effects. First activated sludge systems tended to be single stage systems, but activated sludge process designs continued to develop for example in order to meet the removal demand for nutrients. Nowadays various-stage activated sludge systems as well as activated sludge system accompanied with chemical and physical unit operations and processes are utilized in the advanced wastewater treat-

ment. (Ardern & Lockett 1914; Rittmann & McCarty 2001; Metcalf & Eddy, Inc., 2003)

3.1 Conventional Process Configurations

Basic goal of activated sludge wastewater treatment process is degradation of the contaminants by aerobic microbes, mainly bacteria and protozoa, of the sludge. Activated sludge microbial ecology will be further discussed in Chapter 3.2. A basic activated sludge system always includes the following unit operations, processes and equipment, also presented in
(Karttunen et al. 2004; Seviour & Nielsen 2010).

- Primary clarification, although this may not be necessary in the small scale systems.
- Aeration, where wastewater is lead in contact with the treating biomass.
- Clarification, where the treated water and the consisted biomass are separated.
- Sludge recycling unit operation, where part of the clarified sludge is returned into the aeration. This is fundamental for maintaining the desired sludge concentration in the aeration tank.
- Unit operation for removing the excess sludge from clarification.
- Aeration equipment for producing air (and oxygen) needed in the aeration.
- Stirring equipment for the aeration, if the aeration itself does not provide efficient mixing to maintain the system in a constant state of suspension.

The original conventional activated sludge systems have long narrow aeration tanks in which the wastewater enters at one end and exits through the other. These systems have significant plug flow character. System with plug flow characteristics has also limitations. High contaminant concentrations at the head of the aeration tank may lead to complete depletion of dissolved oxygen and anoxic conditions, which inhibits the activity of contaminant degrading microbes. Contaminants may also have toxic effects for activated sludge microbes. High local concentrations can be avoided by distributing the influent evenly along the length of aeration tank, which is called step aeration or step feeding. Another solution to spread the influent evenly is the completely mixed activated sludge process, or continuously stirred tank reactor (CSTR) as commonly referred in literature. However, categorising processes to plug flow or completely mixed is imprecise, because the necessary aeration of plug flow process will inevitably produce some mixing, and total complete mixing in full scale applications is unlikely to be achieved in practise. (Rittmann & McCarty 2001; Seviour & Nielsen 2010.)

The contact stabilization process permits high efficiency treatment to occur in a significantly reduced total reactor volume. Wastewater is mixed with activated sludge in

a contact reactor having a short detention time, allowing readily biodegradable compounds to be oxidized or stored inside the cells. After settling, the concentrated sludge is sent to a stabilization tank, where the majority of the oxidation actually occurs. (Rittmann & McCarty 2001)

One of the recent innovations is to add an anoxic selector tank prior to the aerobic reactor. Anoxic conditions in the selector produce fatty acids, that certain microbes are able to store in the form of glycogen or polybetahydroxybutyric acid (PHB). These same microbes, which are good at forming compact floc particles, benefit from the storage material when they enter into the oligotrophic environment of the aeration tank. Thus, the function of the selector is to select or change the ecology of the activated sludge towards organisms with good settling characteristics. (Rittmann & McCarty 2001) Flow graphs of different conventional activated sludge process configurations are presented in Figure 3.1.

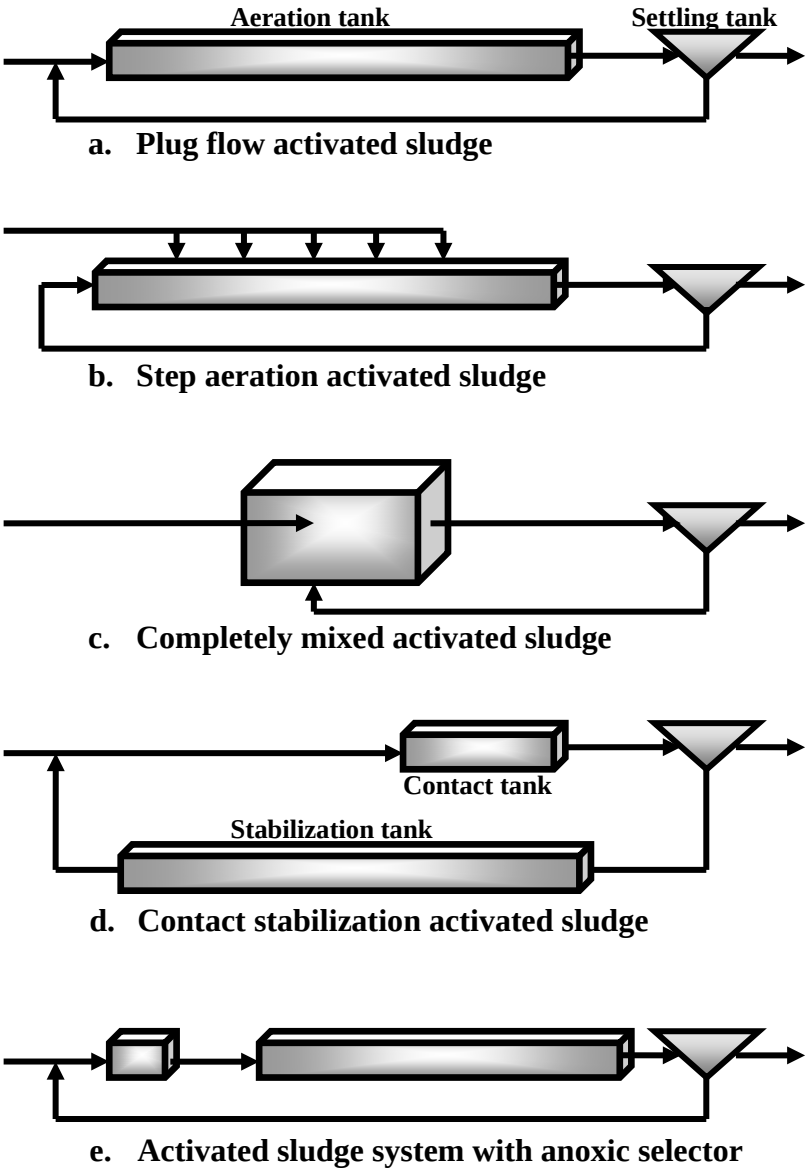

Figure 3.1. *Flow graphs of different conventional activated sludge systems (Rittmann & McCarty 2001).*

3.2 Microbial Ecology

Most of the activated sludge consists of living, dead or lag phase microbes (or substances produced or related to them), and the variety of organisms present is extremely wide. These organisms take up the wastewater contaminants and use them for growth or energy supply. Fundamental organisms are prokaryotes (bacteria) and eukaryotes (algae, protozoa, crustacea, nematodes and rotifera). Viruses and bacteriophage, which are bacterial viruses, also exist in activated sludge. Fungi and yeasts are also present, but they seldom are important members of the activated sludge community. (Rittmann & McCarty 2001; Karttunen et al. 2004; Seviour & Nielsen 2010.)

Because of great variety of different organisms and the great competition for energy resources in activated sludge, even minor changes in treatment process or influent composition may result in major changes in the microbial population (Rittmann & McCarty 2001). Although basic principles and functions of activated sludge process are well known, it has been very difficult to measure, define or utilize the best microbial community with the best performance. Typical failures in activated sludge systems, such as bulking and foaming, are at least partly caused by a lack of understanding the composition of microbial communities in those systems. Diversity of microbial populations is said to be connected with process stability and pollutant removal, although the problem is that the diversity cannot be systematically manipulated. (Rowan et al. 2003; Akarsubasi et al. 2005; Siripong & Rittmann 2007; Pholchan et al. 2010.)

3.2.1 Flocs

Most of the activated sludge organisms are held together within flocs by naturally produced organic polymers and electrostatic forces. Whether the organism is located inside the floc or on its surface or freely suspended in the bulk liquid is crucial to the faith of the organism and eventually to the whole process and the solid-liquid separation. It will determine whether the population is retained within the reactor or washed out within the clarified liquid phase, finally impairing the effluent quality. Most bacteria inside or on the surface of the floc are perceived as metabolically active. The location of the organism may also influence its access to nutrients and oxygen, as well as its sensitivity to toxic compounds. (Wilén et al. 2008a; Rittmann & McCarty 2001; Seviour & Nielsen 2010.)

Only a minor fraction (5-20%) of the organic matter in the activated sludge floc is made up from bacterial cells (Wilén et al. 2008b). An activated sludge floc can be viewed as a complex heterogeneous structure, composed of aggregates of bacteria and other organisms that are embedded in a polymeric matrix or gel. This matrix is often referred to as extracellular polymeric substances (EPS), and it is responsible for more than 50% of the flocs organic matter. The EPS are typically made up of proteins, humic substances, carbohydrates, nucleic acids and lipids, and they are produced by the microorganisms either by cell lysis or by active transport. Also organic fibres, organic particles from the wastewater and inorganic components may be integrated into the matrix.

Most bacteria exist as microcolonies or microflocs that are bound into the floc structure, but some are present as single cells or as filamentous bacteria. The bacterial community composition and activity of the floc, as well as floc properties such as size distribution and morphology, can change as a result of the process type and even during operation. Floc stability is often influenced by environmental stresses such as sudden temperature or pH fluctuations, or by poor bioflocculation. (Jin et al. 2003; Wilén t al. 2008b; Seviour & Nielsen 2010.)

According to the study of Wilén et al. (2008a), lower temperature was found to cause weaker floc structure and higher concentrations of proteins and carbohydrates in the EPS matrix. In the same study, sludge containing higher concentrations of iron had poorer settling and compaction properties but better floc stability. Also increased organic loading had positive effects on the solid– liquid separation properties of the activated sludge flocs.

3.2.2 Prokaryotes

By mass, the dominant members of the activated sludge community are heterotrophic bacteria. Heterotrophic bacteria are also the primary consumers of organic wastes, although with certain organic particles protozoa may be involved as well. The majority of activated sludge bacteria are Gram-negative. Principal identified genera are *Pseudomonas, Arthobacter, Comamonas, Lophomonas, Zoogloea, Sphaerotilus, Azotobacter, Chromobacterium, Achromobacter, Flavobacterium, Bacillus, Nocardia, Bdellovibrio and Mycobacterium.* Bacteria taking part of the nitrogen removal, nitrifying bacteria, are mainly from *Nitrosomonas* and *Nitrobacter* genera. (Rittmann & McCarty 2001; Karttunen et al. 2004.)

Filamentous bacteria, such as *Alphaproteobacteria* (‘*Nostocoida’*-like), the *Gammaproteobacteria* (*Thiothrix*), the *Actinobacteria* (*Candidatus ‘Microthrix’*, *Mycolata*) and the *Chloroflexi,* have a divaricate role in activated sludge process. Filamentous bacteria constitute the backbone of the floc with fibres and bacterial colonies, but on the other hand they can cause problems when extended outside the floc. This typically causes bulking and foaming problems that can prevent adequate flocculation and settling of the biomass, leading to a carryover of solids with the final effluent. (Keiding & Nielsen 1997; Wilén et al. 2008b; Nielsen et al. 2009)

Viruses are also a part of the microbial system of activated sludge, although their roles have not yet been completely clarified. Study of Otawa et al. (2006) suggested that the concentrations of viruses in activated sludges were similar or higher than the concentrations in natural environments. The role of bacterial viruses, bacteriophage, is not broadly documented, but there are findings suggesting that bacteriophage may play a significant role determining the structure and function of bacterial communities in activated sludges. Since there are multiple bacterial species present, if one species is decimated by a phage, another species can replace it rapidly, without any detectable interference for treatment efficiency. This is a fine example of redundancy in microbial eco-

systems, and probably one of the main reasons why activated sludge process works so reliably. (Rittmann & McCarty 2001; Barr et al. 2010)

3.2.3 Eukaryotes

The most common activated sludge predators, a.k.a. organisms that use other organisms for energy and growth, are protozoa. Many species of protozoa have been identified, and the total number is said to be approximately 50 000 cells/mL, or even 5% of the total dry weight. Protozoa require a longer sludge retention time than aerobic heterotrophic bacteria, and prefer dissolved oxygen concentration above 1.0 mg/L. (Ratsak et al. 1996; Rittmann & McCarty 2001; Metcalf & Eddy, Inc., 2003.) Protozoa are not the primary consumers of organic wastes in the activated sludge process, but for long time they have been known as useful indicators of process performance. The effectiveness of protozoa is due to the fact that they feed on dispersed bacteria, that otherwise could cause problems in the process. The presence of ciliate protozoa reflects an effectively working process and an increase in effluent quality, and especially ciliates that attach to the floc particles with a stalk are the best indicators of a stable sludge. Protozoa are known to be highly sensitive to toxic chemicals; hence a healthy protozoa population also indicates that the wastewater is relatively free of toxics. Another fact promoting the role of protozoa as useful indicators is that their presence and activity is readily observed with a low-powered microscope. (Salvadó et al. 1995; Ratsak et al. 1996; Rittmann & McCarty 2001.) In addition to being useful indicators, protozoa also participate in the cycling of carbon and nutrients (phosphorus and nitrogen). This has been studied in some detail in aquatic systems and activated sludge mixed liquor, and to a lesser extent in the soil. (Akpor et al. 2008.)

Also rotifers, nematodes and other multicellular organisms are often found in activated sludge systems. Their roles have not yet been well defined, but because they normally are present at longer sludge retention times, they are used as indicators. Multicellular predators are able to ingest whole groups of bacteria, and are commonly seen within the microbial flocs chewing of bits and pieces of the floc particles. (Rittmann & McCarty 2001; Metcalf & Eddy, Inc., 2003.)

3.3 Design and Operating Parameters

Activated sludge process is based on the mixing of microorganisms and wastewater under aerobic conditions (Arden & Lockett 1914). Microbial communities in activated sludge systems as well as whole functionality of the system are likely to be affected by factors such as the influent characteristics and volume, the environmental conditions, system design and operation. Microorganisms perform oxidation/reduction reactions that generate the energy and reducing power required to maintain life and construct new cells. For example, organic substances act as electron donors for heterotrophic bacteria, NH_4-N is an inorganic electron donor for nitrifying bacteria, NO^-_3 is an electron acceptor for denitrifying bacteria, and PO_4^{3-} is a nutrient for all microorganisms. In literature,

the term substrate typically refers to the primary electron donor that limits the growth rate of the biomass. Usually in activated sludge systems the primary electron donors are organic substances. The connection between the active biomass and primary substrate is the key factor needed for understanding and controlling biological wastewater treatment systems. Naturally activated sludge process parameters are highly related to kinetics of biological growth, substrate utilization, substrate removal and mass balance. (Rittmann & McCarty 2001.)

The relationship most frequently used to present bacterial growth kinetics is so called *Monod equation*, Equation 3.1.

$$\mu_{syn} = \left(\frac{1}{X_a}\frac{dX_a}{dt}\right) = \mu_{max}\frac{S}{K_s+S} \tag{3.1}$$

where

X_a = active biomass concentration (g/m^3)
μ_{syn} = specific growth rate due to synthesis (1/d)
μ_{max} = maximum specific growth rate (1/d)
S = growth limiting substrate concentration in solution (g/m^3)
K_s = substrate concentration giving one half the maximum growth rate, half velocity constant (g/m^3)

The *Monod equation* can be expressed in various forms depending on the special interest or purpose of use. Because in activated sludge systems the ultimate interest is substrate (BOD) removal, and because biomass growth is fueled by substrate utilization, the rate of substrate utilization can be regarded to as a basic rate, and cell growth controls the concentration of growth limiting substrate. Then, the *Monod equation* can be expresses in a form presented in Equation 3.1. (Rittmann & McCarty 2001).

$$r_{ut} = -k\frac{SX_a}{K_s+S} \tag{3.2}$$

where

r_{ut} = rate of substrate utilization (g/m^3*d)
k = maximum specific rate of substrate utilization (g substrate/g biomass*d)
S = growth limiting substrate concentration in solution (g/m^3)
X_a = active biomass concentration (g/m^3)
K_s = substrate concentration giving one half the maximum growth rate, half velocity constant (g/m^3)

Parameters used to design and operate activated sludge processes vary from totally empirical to those that are soundly based on fundamental theories. Many equations and relationships that are developed for expressing microbial kinetics factors can be used for process designing, but their full utilization is difficult because of many constants that may not be easily and positively determined. Therefore more practical equations have been developed to help the design. (Rittmann & McCarty 2001; Karttunen et al. 2004; Pholchan et al. 2010.) Following subchapters review the major design and operating parameters of an activated sludge system.

3.3.1 Food to Microorganism Ratio

The food to microorganism ratio (F/M ratio) is widely used for characterise process design and operation conditions because it is simple and relies on relatively easy measurements that can be conducted on the WWTP. The F/M ratio defines how many mass units of substrate are available per mass unit of total suspended solids (TSS) or volatile suspended solids (VSS) in the aeration tank. Typically in professional literature, activated sludge TSS is referred to as mixed liquor suspended solids (MLSS), and VSS is referred to as mixed liquor volatile suspended solids (MLVSS).

The F/M ratio can be calculated using Equation 3.2,

$$\frac{F}{M} = \frac{Q_{inf} S_{inf}}{V\ X} \tag{3.3}$$

where

F/M = F/M ratio (gBOD/gMLVSS*d)
Q_{inf} = Influent wastewater stream flowrate (m^3/d)
S_{inf} = Influent wastewater BOD or COD (mg/L)
V = Volume of the aeration tank (m^3)
X = MLSS or MLVSS in the aeration tank (mg/L)

The design and operation of the activated sludge process are highly affected by the decided organic loading. According to Rittmann & McCarty (2001), the four major loading modifications are referred to as conventional loading, modified aeration, high rate and extended aeration. Typical values of BOD F/M ratio vary from 0.04 g BOD/gMLVSS*d (extended aeration) to 1.0 g BOD/gMLVSS*d (high rate process). Also MLSS may be used instead MLVSS. (Rittmann & McCarty 2001; Metcalf & Eddy, Inc., 2003; Pholchan et al. 2010.)

3.3.2 Hydraulic and Solids Retention Time

Hydraulic retention time (HRT) is a measure of the average period of time during which the water remains in the whole activated sludge system, and calculated according to Equation 3.3.

$$HRT = \frac{V_{sys}}{Q_{inf}} \tag{3.3}$$

where

V_{sys} = System volume (m^3)
Q_{inf} = Influent flowrate (m^3/d)

More critical parameter for activated sludge design than HRT is solids retention time (SRT). The SRT represents the average period of time during which the sludge remains in the system (aeration + settling). The SRT has an impact on the sludge's physical and biological characteristics. Activated sludge is a complex ecological system including both the faster growing primary consumers of influent substrate, and the slower growing secondary organisms and predators that live of the primary consumers. Operation at long SRT allows the accumulation of more slowly growing organisms that would be washed out from the reactor with short SRT. As previously mentioned, some slowly growing predators like stalked ciliates are beneficial for the system, but some relatively slow growers like filamentous bacteria can cause operational problems such as bulking and foaming. Also nitrifying bacteria are slow growers that can exist in the sludge only with long SRT. Thus, the SRT has to be carefully considered when designing or operating an activated sludge WWTP. (Rittmann & McCarty 2001; Clara et al. 2005.)

All the parameters that comprise SRT can be measured accurately and consistently, but an excellent way to examine and estimate the SRT and its influence is microscopic examination. Slow growing organisms such as ciliates and rotifers are easily observed and recognized with a basic phase contrast microscope using 100-times magnification. In a properly functioning WWTP, the protozoa population remains stable and typical for the WWTP in question. Sudden alterations in loading, toxic shocks or lack of oxygen cause population changes or death of the protozoa, which is easily observed by a skilled microscope user before the changes affect the removal efficiency. (Rittmann & McCarty 2001; Karttunen et al. 2004.) The relative predominance of microorganisms versus F/M ratio and SRT is presented in Figure 3.2.

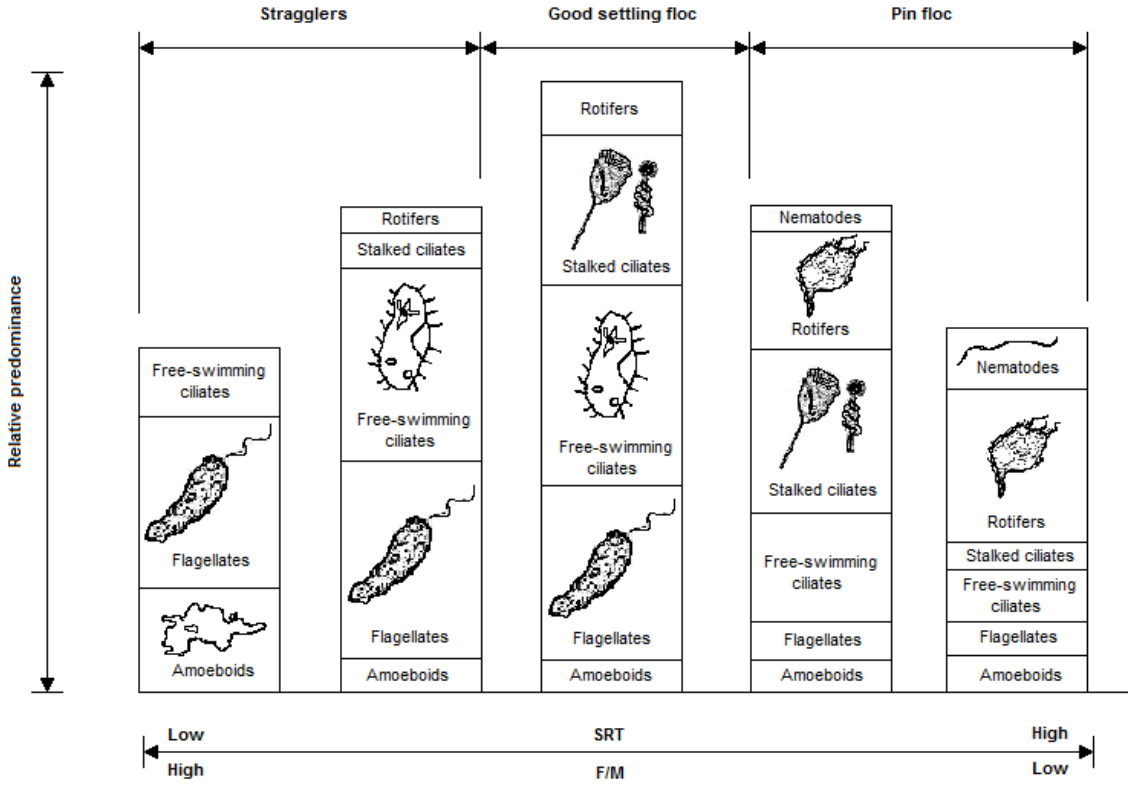

Figure 3.2. *The relative predominance of microorganisms versus F/M ratio and SRT (Metcalf & Eddy, Inc. 2003).*

The SRT can be calculated using Equation 3.4,

$$SRT = \frac{XV_{sys}}{Q_{eff}X_{eff}+Q_wX_w} \qquad (3.4)$$

where

X, X_{eff} , X_w	=	MLSS or MLVSS of the sludge, effluent and waste sludge (mg/L)
V_{sys}	=	System volume (m^3)
Q_{eff}	=	Effluent flowrate (m^3/d)
Q_w	=	Waste sludge flowrate (m^3/d)

Typical SRT values for conventionally loaded treatment systems are in the range of 4 to 10 days. Extended aeration WWTPs generally have much longer SRT, in the range of 15 to 30 days or even more. The modified aeration processes have short SRTs, from 0.2 to 0.5 days. (Rittmann & McCarty 2001; Clara et al. 2005.)

3.3.3 MLSS, Recycle Ratio and Sludge Volume Index

One major parameter affecting all previous parameters of activated sludge is MLSS concentration. The MLSS concentration is a complex parameter. Increasing the MLSS leads to a demand for a smaller aeration basin and lower construction costs. On the other hand, greater MLSS demands larger settling tanks and more intense aeration. Increasing MLSS also requires higher recycle rate of sludge from the settling tank back to the aeration tank, which demands more pumping. (Rittmann & McCarty 2001.)

The MLSS concentration depends upon many factors, such as sludge settling characteristics and the design of the settling tank. One of the factors affecting the MLSS concentration is recycle ratio, which can be calculated according to Equation 3.5. Generally the higher recycle ratio, the higher MLSS concentration in the aeration.

$$R = \frac{Q_r}{Q_{inf}+Q_r} \tag{3.5}$$

where

R = Recycle ratio
Q_r = Recycle flow rate (m^3/d)
Q_{inf} = Influent flowrate (m^3/d)

Naturally the best effluent quality can be achieved with properly settling sludge. Settling is affected by the geometry of the settling device, the concentration of MLSS, the sludge volume, the temperature as well as the floc structure (Jin et al. 2003). One of the tools giving information on the sludge settleability and quality is sludge volume index (SVI). For SVI test, activated sludge from aeration basin is left to settle in 1 L cylinder for 30 min. The original MLSS concentration and the volume of the settled sludge after 30 min are measured. (Rittmann & McCarty 2001.) SVI (mL/gMLSS) is defined according to Equation 3.6.

$$SVI = \frac{V_{30} \cdot 1\,000 \, {}^{mg}/_{g}}{MLSS \cdot V_s} \tag{3.6}$$

where

V_{30} = Volume of the settled sludge after 30 min (mL)
$MLSS$ = The original mixed liquor suspended solids concentration (mg/L)
V_s = Cylinder volume (L)

SVI value of 100 mL/g is considered as typical good settling sludge, although values below 100 mL/g are desired. SVI values above 150 mg/L express filamentous sludge or bulking. (Rittmann & McCarty 2001; Metcalf & Eddy, Inc., 2003.)

3.4 Fundamental Factors

Aeration, temperature and pH are fundamental factors that affect both the microbiological and physical circumstances of the activated sludge system. Even though especially temperature and pH are more or less dependent on the influent characteristics, they can be adjusted and controlled even during separate processes, driving the processes towards desired outcome. Naturally, the extent and methods of those adjustments affect the expenses of the WWTP. (Rittmann & McCarty 2001; Holenda et al. 2008; Liao et al. 2011.)

3.4.1 Aeration

Aeration has two purposes in activated sludge systems: oxygen supply and mixing. Enough oxygen has to be supplied for the microbial metabolism, so organic matter can be degraded and ammonium converted to nitrate. Theoretically the volume of oxygen supplied to the aeration basin has to be equal to the volume of oxygen required by the microorganisms. In practise, the dissolution of oxygen from gas to the liquid phase is relatively low, so microbes are able to utilize only a small volume of supplied oxygen. (Metcalf & Eddy, Inc., 2003; Holenda et al. 2008.)

When oxygen limits the growth, filamentous microorganisms may predominate, causing formation of porous flocs, deteriorated settling and poor sludge quality. It is generally suggested that the minimum of 1.5 - 2 mg/L dissolved oxygen (DO) concentration needs to be maintained in the reactor in order to suppress the growth of filamentous organisms. Nitrifying bacteria may prefer higher DO, and denitrifying bacteria require anaerobic conditions, thus they are more discussed in Chapter 3.5. (Wilén & Balmér 1999; Rittmann & McCarty 2001; Metcalf & Eddy, Inc., 2003; Holenda et al. 2008; Gao et al. 2011.)

Mixing of reactor contents is required for keeping the MLSS in suspension and well distributed in the aeration basin. Oxygen is delivered into the activated sludge through diffused or mechanical aeration. In diffused aeration, compressed air or pure oxygen is bubbled from submerged diffusers, and the bubbles create mixing while rising into the surface. In mechanical aeration, a mechanical mixer causes oxygen transfer from the atmosphere above. Mixing and aeration have to be properly designed and maintained to meet the oxygen requirements as well as the economical factors. Too intense aeration may also damage the flocs and cause poor sludge quality. (Rittmann & McCarty 2001; Holenda et al. 2008.)

Dissolution of oxygen into a liquid is depending on many factors, such as process temperature, air pressure and other dissolved substances e.g. salinity. In addition especially in WWTPs, oxygen solubility is affected by concentration of oxidable organic material in wastewater, activated sludge suspension, as well as the geometry of the aeration basin. Solubility of transferred oxygen in particular process can be evaluated in laboratory experiments. However, utilizing the results in a full scale plant may require more measurement and modelling. (Rittmann & McCarty 2001; Holenda et al. 2008.)

3.4.2 Temperature

Temperature is an important factor affecting biomass activity, as well as selection and survival of microorganisms which are critical for maintaining efficient biological wastewater treatment. In general, the optimal growth temperature range for a particular microorganism is fairly narrow, although most microorganisms are able to survive within much broader limits. Typically the growth rate is much more affected if temperature is below the optimum than above it. Until the optimum temperature is reached, the growth rate is said to double with approximately every 10 ^{o}C increase. According to their optimal temperature range, microorganisms can be classified as psychrophilic, mesophilic, or thermophilic, as presented in Table 3.1. (Sedory & Stenstrom 1995; Metcalf & Eddy, Inc., 2003; Lippi et al. 2009)

Table 3.1. *Temperature classification of biological processes (Metcalf & Eddy, Inc., 2003).*

Type	Temperature range [^{o}C]	Optimum range [^{o}C]
Psychrophilic	10 - 30	12 - 18
Mesophilic	20 - 50	25 - 40
Thermophilic	35 - 75	55- 65

Temperature not only influences the metabolic activity and microbial ecology, but also has a strong effect on such factors as gas transfer rate and sludge settling characteristics. The solubility of oxygen into the liquid increases as temperature decreases, meaning that less aeration is needed to supply the required oxygen. In many cases, the sludge settleability has been reported to be worse in cold temperatures, although also results indicating no variation between different thermal seasons have been reported. (Rittmann & McCarty 2001; Metcalf & Eddy, Inc., 2003; Jones & Schuler 2010.)

3.4.3 pH

The pH of the environment is also a key factor in the growth and selection of the activated sludge microorganisms. Most of the bacteria cannot tolerate pH levels below 4 or above 9. Optimal pH is generally between 6.5 and 7.5. (Rittmann & McCarty 2001; Metcalf & Eddy, Inc., 2003.) Reactions such as the first step of nitrification (ammonium oxidation) and simultaneous chemical precipitation with metal salts produce acidity and consume alkalinity, which leads to pH decrease. In order to maintain the pH between optimum range, alkalinity (e.g. $CaCO_3$) needs to be added into the process. (Sherrard 1976; Rittmann & McCarty 2001.)

3.5 Nitrogen Removal

As discussed previously, removal of nitrogen and phosphorus from wastewaters has become increasingly important because of eutrophication of the receiving waters. Typical municipal wastewater includes on average 40 mg/L of total nitrogen. In a conventional aerobic wastewater treatment process, only 10-15% of wastewater nitrogen compounds are bound into the sludge. If nitrogen removal is desired, a specific process design and control are required. Nitrogen in wastewater is mainly present as ammonium, which is first oxidized to nitrite and then nitrate by autotrophic nitrifying bacteria. In some conditions, WWTPs with relatively low organic loading are able to spontaneously nitrificate. Especially in older WWTPs, nitrification is considered sufficient for nitrogen removal, because nitrate does not consume oxygen in the receiving waters, and is not toxic to aquatic life like ammonium. The transformation of ammonium into nitrate can be close to 99%, but proper nitrogen removal can be considered only when also denitrification is conducted. Denitrifying bacteria are heterotrophs that reduce nitrate into nitrogen gas, which is released into air. Denitrification occurs at anoxic conditions without dissolved oxygen. Nitrate acts as electron recipient, while organic carbon compounds are electron donors. In Finland, nitrogen removal from municipal wastewaters can be even 85% at best, during a period when wastewater temperature is at the highest. Generalized nitrogen transformations in biological treatment processes are as presented in Figure 3.3. (Rittmann & McCarty 2001; Metcalf & Eddy, Inc., 2003; Karttunen et al. 2004; Pai et al. 2004.)

Nitrification and denitrification set demands for activated sludge process design. Nitrifiers are slow growers, so if plenty of organic carbon is available, they lose the competition for space to the faster growing aerobic heterotrophs. Thus, nitrifiers prefer lower proportion of carbon to nitrogen (C/N ratio). Nitrification can be executed in one or two sludge systems. In two sludge system, organic matter is oxidized in the first stage and ammonium in the second. In one sludge system, both the oxidation of organic material and ammonium take place in the same aeration basin. In order to reach the low C/N ratio, one sludge systems have a very low organic loading and high MLSS concentration, which is maintained with long SRT. Aeration basin may also function as a plug flow reactor, causing the circumstances beneficial for nitrification to take place in the end of the basin. (Rittmann & McCarty 2001; Karttunen et al. 2004; Pai et al. 2004; Wijeyekoon et al. 2004; Islam et al. 2009.)

The impact of temperature for treatment efficiency can be significant especially for nitrogen removal. Nitrification is sometimes considered almost impossible for wastewaters with temperature below 5 ^{o}C. Nitrification in cold wastewaters requires larger aeration basin volumes and longer SRT to maintain enough sludge volume for nitrification. Long SRT, for one, benefits the growth of filamentous bacteria that reduce sludge quality. In Finland, seasonal variation in WWTPs process temperatures is quite large, approximately on a range of 4 ^{o}C to 25 ^{o}C. According to EU-legislations, requirement for nitrification takes place only during a time period when process tempera-

ture is above 12 ^{o}C. However, normally in Finland nitrification is executed year-round, and Finnish WWTPs are typically run with extended aeration (SRT > 15 d). (Sedory & Stenstrom 1995; Rantanen et al. 2003, Metcalf & Eddy, Inc., 2003; Karttunen et al. 2004; Lippi et al. 2009)

There are many process configurations for nitrification-denitrification. Denitrification as separate process can occur before or after nitrification, although external carbon may have to be added if proper nitrogen removal is desired. In combined nitrification-denitrification systems, anaerobic and aerobic zones alternate in same aeration basin. While denitrifying bacteria use direct wastewater as their carbon source, recycled sludge is returned into aerobic zone in order to maintain high MLSS. The benefits of combined process include smaller aeration basin volumes, no external carbon requirement, no settling between processes, better sludge settling and better interruption resistance. (Rittmann & McCarty 2001; Metcalf & Eddy, Inc., 2003; Karttunen et al. 2004; Pai et al. 2004; Wijeyekoon et al. 2004; Islam et al. 2009.)

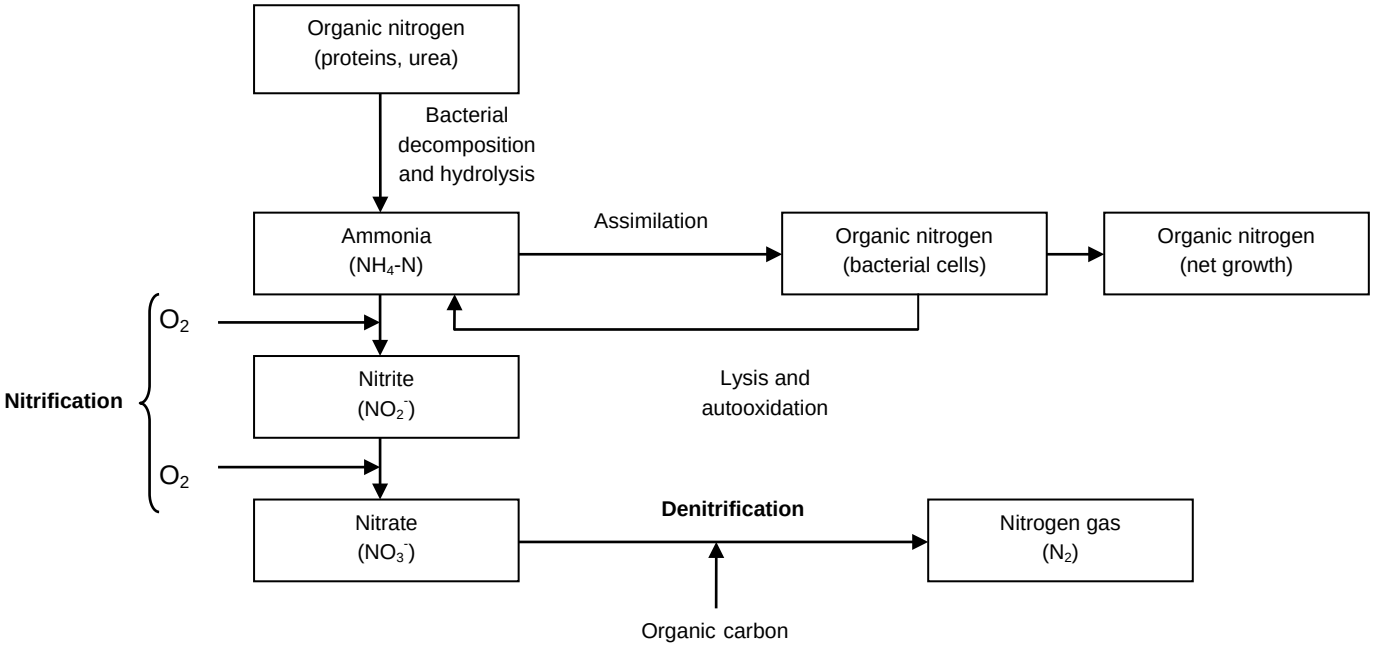

Figure 3.3. *Nitrogen transformations in biological treatment processes (Metcalf & Eddy, Inc. 2003).*

3.6 Phosphorus Removal

Typical municipal wastewater includes on average 7 mg/L of phosphorus. Usually phosphorus is present in organic form, orthophosphate or complex phosphate. Phosphorus is usually the limiting nutrient for algal growth in freshwater environments, and stimulates the growth of for example toxic cyanobacteria (blue-green algae). In conventional aerobic wastewater treatment, only 10-30% of the wastewater phosphorus is bound into the sludge. The most usual technique for removing phosphorus from municipal wastewaters is chemical precipitation, but also biological phosphorus removal is executed on many WWTPs around the world. (Ekholm & Krogerus 1998; Mainstone & Parr 2002; Finland's Environmental Administration 2011; Karttunen et al. 2004.)

Chemical precipitation is usually executed in three stages; mixing, flocking and settling. Added precipitation chemical reacts with the soluble phosphorus forming a phosphate deposit. Typical phosphate precipitation chemicals for municipal wastewaters are aluminium or iron salts, such as aluminium sulphate [$Al_2(SO_4)_3$], polyaluminium chloride [PAC], ferrous sulphate [$FeSO_4$], ferric sulphate [$Fe_2(SO_4)_3$], or ferrous chloride [$FeCl_3$]. Ferrous sulphate is typically added 80-130 g/m^3 wastewater. Some of the precipitation chemicals are pH-sensitive and may require acid or lime addition. For acidic wastewaters, lime may also be used for phosphate precipitation. (Finland's Environmental Administration 2011; Rittmann & McCarty 2001; Karttunen et al. 2004.)

Chemical precipitation can be executed separately or combined with biological wastewater treatment. In combined precipitation-biological treatment systems, the three different process configurations are pre-precipitation, after precipitation and simultaneous precipitation. When pre-precipitation is used, the phosphate deposit mixes with primary settled sludge. In after-precipitation systems, the chemical deposit remains relatively pure, and in simultaneous precipitation the deposit mixes with the activated sludge. (Finland's Environmental Administration 2011; Rittmann & McCarty 2001.)

In biological phosphorus removal, the wastewater phosphorus is included into cell biomass, which subsequently is removed from the process with excess sludge. The principal advantages of biological phosphorus removal on chemical precipitation are reduced chemical costs and smaller sludge production. (Rittmann & McCarty 2001; Metcalf & Eddy, Inc., 2003; Karttunen et al. 2004.) The group of microorganisms that are largely responsible for phosphorus intake are known as the polyphosphate accumulating organisms (PAOs). The goal of biological phosphorus removal is select PAOs, induce them to store phosphate as intracellular polyphosphate and collect them in excess sludge when they are rich in biomass. The process requires both anaerobic and aerobic zones. In anaerobic zone PAOs use energy from stored polyphosphates, assimilate acetate and produce intracellular storage products like polyhydroxybutyrate (PHB) with the concomitant release of phosphorus. In the aerobic zone energy from the stored PHB is utilized for cell growth and high polyphosphate storage. As a portion of biomass is collected, the stored phosphorus is removed from the process. (Lötter 1985; Wentzel et al. 1990; Rittmann & McCarty 2001; Metcalf & Eddy, Inc. 2003.)

3.7 Problems in Activated sludge Process

The most fundamental requirement for properly functioning activated sludge process is a well settling and compacting sludge floc, which allows a good solid-liquid separation. Settling is important for minimizing the effluent suspended solids concentration. The sludge must also compact well so it can be successfully returned from settling tank to the aeration basin. (Rittmann & McCarty 2001.) Poor solid-liquid separation occurs occasionally at most WWTPs, causing poor effluent quality and operation problems. There are several reasons for this, such as filamentous bulking sludge, bulking due to excessive extracellular polymeric substances (EPS) production or formation of small flocs and dispersed biomass. (Wilén et al. 2008a) The typical solid-liquid separation problems encountered in activated sludge operation are presented in Table 3.2.

Table 3.2. *Typical solid liquid separation problems on activated sludge WWTP (Rittmann & McCarty 2001; Jenkins et al. 2003).*

Separation problem	Cause of problem	Effect of problem
Bulking	Filamentous organisms extend from flocs into the liquid, present bridges between flocs and interfere the separation	High SVI with clear supernatant. Overflow of sludge blanket and hydraulically overloaded solids handling processes
Viscous bulking	Microorganisms produce large amounts of extracellular slime, which can even impart a jelly like consistency	Reduced separation capacity. Can result in overflow of the sludge blanket or formation of viscous foam
Dispersed growth	Microorganisms do not form flocs, but are dispersed, forming only small clumps or being present as single cells	Turbid effluent. No zone settling of sludge
Pinpoint floc	Small, compact, weak, roughly spherical flocs. Larger flocs settle rapidly, smaller ones slowly	Low SVI and turbid, often high TSS effluent
Foaming	Caused by nondegradable surfactants or presence of *Nocardia sp.* and/or *Microthrix parvicella*	Foam floats large amount of TSS to surface, which causes solids overflow into effluent and to the surroundings. Microorganism-caused foams are persistent and difficult to break.
Blanket rising	Denitrification in settling tank releases soluble N_2 gas, which attaches to sludge flocs and causes floc flotation	“Rafts” of sludge collect on the settler surface and may result in solids overflow and turbid effluent

3.7.1 Problems Caused by Lipids

Lipids can cause various problems in wastewater treatment plants, including sludge flotation, filamentous bulking and foaming. In the sewers, lipids, especially mineral oils, tend to coat surfaces and cause maintenance problems. If entered into the receiving waters, lipids may interfere with the living organisms and cause unsightly floating matter and films. (Quéméneur & Marty 1994; Raunkjær et al. 1994; Chipasa & Medrzycka 2006; Chipasa & Medrzycka 2008.)

Many methods have been used to enhance the removal of lipids from wastewater. Although literature indicates that lipids are easily biodegraded, and removed by activated sludge in certain extent, WWTP effluents are still reported to contain lipids. (Dignac et al. 2000; Chipasa & Medrzycka 2008.) Chipasa & Medrzycka (2008) studied the fate of lipids in activated sludge under aerobic conditions. The residual lipid content could not be reduced to values below 300 mg/L from the initial lipid concentration of 2 000 mg/L in synthetic wastewater. The similar limitation was also observed in a study of Keenan & Sabelnikov (2000). Wakelin & Forster (1997) used pure acclimated cultures, and managed to reduce the lipid concentration to as low as 100 mg/L.

New approaches and methods (both biological and physicochemical) are still required to fully understand the behavior of lipids in biological wastewater treatment processes and to enhance their removal, as well as to reduce the problems caused by lipids in WWTPs (Chipasa & Medrzycka 2006). Yuan & Blackall (2002) proposed that optimization of microbial community structure could serve as a new way for improving the performance of biological wastewater treatment systems. The microbial community structure can be optimized by selecting the desired species and optimizing their properties, and also controlling the growth of unwanted organisms. These can be achieved by utilizing different operating conditions and reactor configurations, such as pH, temperature, nutrients, electron acceptor and presence or absence of oxygen.

4 SLUDGE PRODUCTION AND PROCESSING IN ACTIVATED SLUDGE WWTPs

In activated sludge process, biomass (sludge) is produced continuously as bacteria and other microorganisms utilize the substrates in wastewater for energy and reproduction. Part of the sludge is returned into aeration basin in order to maintain the desired MLSS concentration and the right concentration of active microorganisms, but large volumes end up as excess sludge. Excess sludge from municipal WWTP contains the same large variety of pathogenic microorganisms than the wastewater, as well as decomposition of the sludge produces greenhouse gases and malodours. Thus, the sludge has to be properly treated. Treatment of excess sludge may constitute approximately one third of the wastewater treatment plants operating costs, although in addition to being bare waste, excess sludge can offer many beneficial utilization possibilities. It can serve as a source of valuable nutrients for agriculture and as a valuable source of energy usually via biogas production or combustion. In addition, excess sludge typically consists of valuable organic substances such as nucleic acids, enzymes, proteins, and polysaccharides. Thus, it is reasonable that new techniques and methods for sludge utilization and disposal are being constantly studied. (Rittmann & McCarty 2001; LeBlanc et al. 2008; Muga & Mihelcic 2008; Hwang et al. 2008; Rantanen et al. 2008; Santala & Etelämäki 2009; Finland's Environmental Administration 2011; Mondala et al. 2011.)

In developed countries, both domestic and industrial wastewaters are often well treated. Worldwide, the volume of wastewater sludge will rise massively when also developing countries are able to treat their wastewaters properly. Sludge production volumes of selected countries, according to the data reported in the Global Atlas of Excreta, Wastewater Sludge, and Biosolids Management (LeBlanc et al. 2008) are presented in Table 4.1. Advanced and controlled wastewater treatment also offers more accurate data on the volumes of treated wastewater and wastewater sludges produced. The volumes are only estimates based on the information that each country has reported for the Atlas, but they give some guidelines on the sludge production volumes worldwide. (LeBlanc et al. 2008.)

Table 4.1. *Sludge production estimates of selected countries (LeBlanc et al. 2008).*

	Sludge Produced [dry tons/a]	Popula-tion [millions]	Sludge Produced [dry tons/million inhabitants*a]	Connected to Wastewater Treatment [%]
Australia and New Zealand	360 000	26	13 971	n.i.a.
Brazil	372 000	201	1 850	21
Canada	860 000	32	26 524	n.i.a.
China	4 152 400	1 330	3 122	44
Czech Republic	200 000	10	19 585	n.i.a.
UK	1 509 000	62	24 322	n.i.a.
Finland	150 000	5	27 891	83
Germany	2 000 000	82	24 377	n.i.a.
Hungary	120 000	10	12 029	68
Italy	1 000 000	60	16 551	n.i.a.
Japan	2 000 000	127	15 704	71
Mexico	640 000	112	5 690	31
Netherlands	1 500 000	17	90 558	n.i.a.
Norway	86 030	5	18 397	n.i.a.
Portugal	236 703	11	22 106	78
Russia	12 000 000	142	84 558	n.i.a.
Slovakia	54 780	5	10 116	n.i.a.
USA	6 514 000	309	21 105	n.i.a.
SUM	**33 754 913**	**2 546**	n.i.a.	n.i.a.
Average			**24 359**	**57**

n.i.a. = no information available

As seen from the Table 4.1, the higher income countries that have the most advanced infrastructure and wastewater treatment technologies (involving e.g. secondary and tertiary treatments), produce the largest masses of sludge per inhabitant. Also residential density has an important role: e.g. in Finland most of the settlement is centred on urban areas and wastewaters can quite easily be collected for effective treatment.

4.1 Biological Sludge Production

Bacteria are the most important microorganism group in activated sludge wastewater treatment. Therefore understanding the fundamentals of bacterial growth is important for understanding sludge production in activated sludge process. Bacteria are able to reproduce by distribution, sexually or by gemmation. Distribution from one to two cells is the most common way to reproduce. In theory with unlimited food and living space, bacteria could reproduce exponentially. In practise lack of food or living space as well

as growth inhibiting extracellular substances limit the growth. (Liu & Tay 2001; Rittmann & McCarty 2001; Karttunen et al. 2004.)

Bacterial growth can be divided into four main phases, as presented in Figure 4.1. The borders between phases are not exact, but they help to understand the growth and to mathematically model the growth curve. Activated sludge is a mixture of multiple microorganisms with different growth rates. In order to simplify the subject, the growth of a single bacterium species can be observed in batch cultivation, where substrate and space are districted. (Karttunen et al. 2004; Metcalf & Eddy, Inc. 2003.)

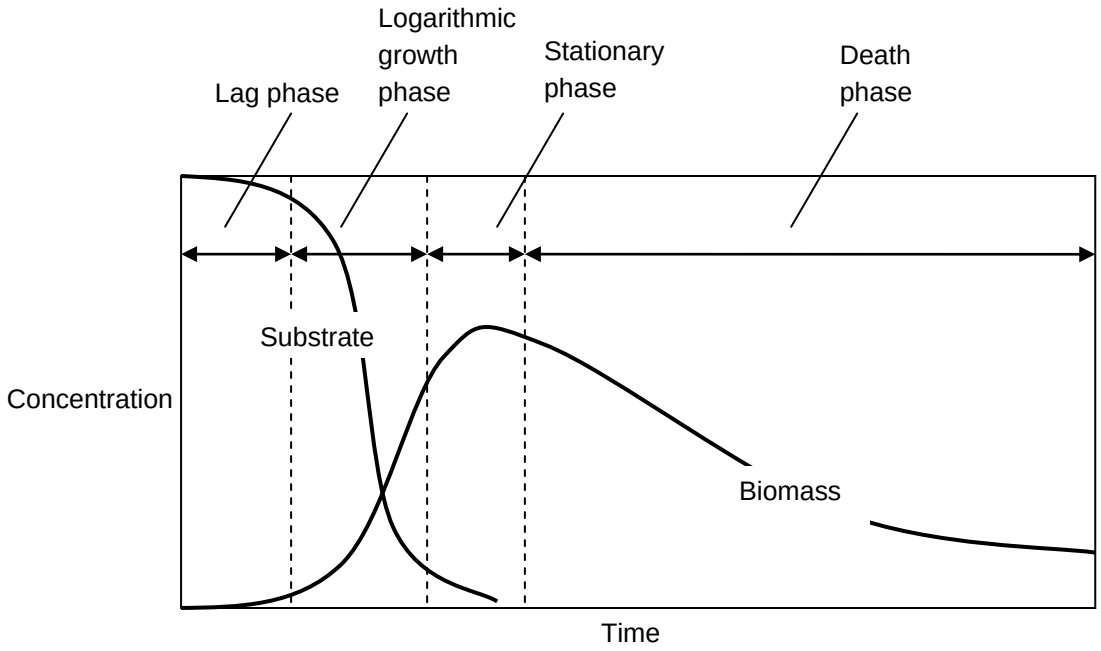

Figure 4.1. *Bacterial growth phases and batch cultivations substrate and biomass concentrations (Metcalf & Eddy, Inc. 2003).*

The four main phases of bacterial growth are (Karttunen et al. 2004.):

1) Lag phase a.k.a. adaptation phase, during which the bacteria adapt theirselves to the environment and substrate. No new cells are formed, but before the first distribution the biomass starts to increase.

2) Logarithmic growth phase, when bacteria reproduce as fast as their growth and nutrient uptake ability allows.

3) Stationary phase, during which the growth decelerates. Substrate or individual nutrient has become a limiting factor, and population size remains constant.

4) Death phase a.k.a. endogenous phase, when external substrate has run out, and biomass is used as food and nutrition. Death rate exceeds the growth rate.

Most of the activated sludge WWTPs operate as continuous processes. The growth phase of the activated sludge microbes is depending on the relation between organic loading and biomass concentration, as presented in Figure 4.2.

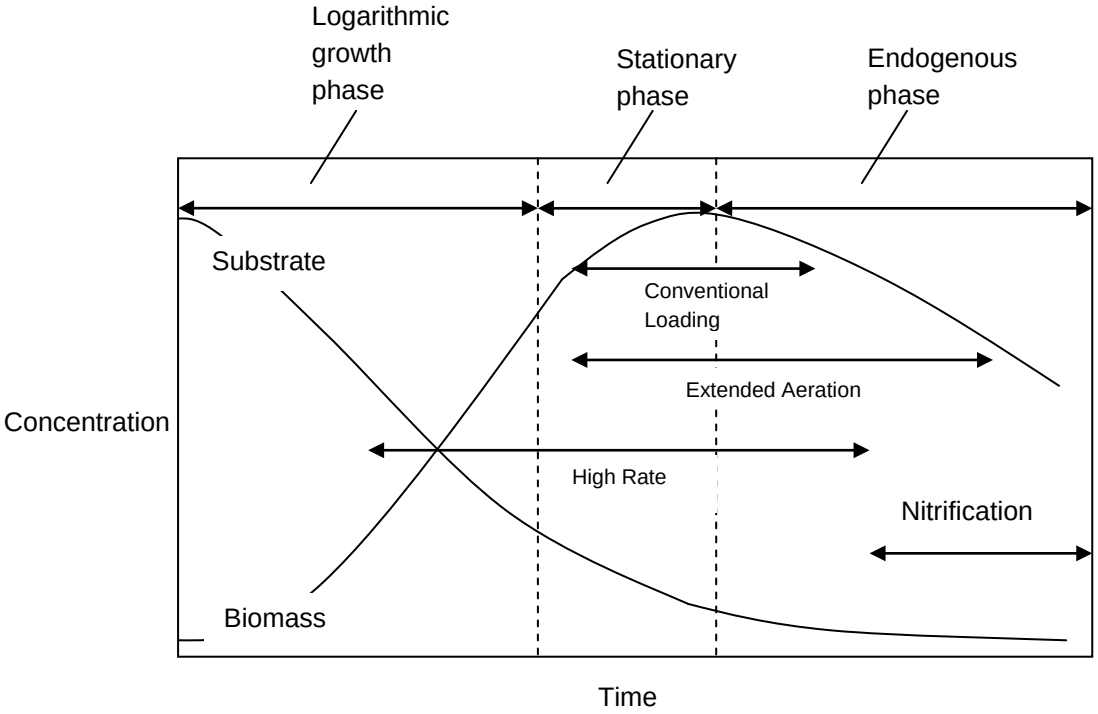

Figure 4.2. *Bacterial growth phases and loading modifications (Karttunen et al. 2004).*

Activated sludge process with short SRT and high F/M ratio is on logarithmic growth phase, and sludge production is relatively high. Compared to high rate, extended aeration processes produce smaller volumes of new biomass because the formed sludge also partially degrades releasing the required nutrients into wastewater. The capability of bacteria to stick together and form flocs is at the best during the stationary phase. If the F/M ratio is too low, the floc will degrade. (Karttunen et al. 2004.)

4.2 Sludge Characteristics

In addition to sludge produced during the biological treatment (secondary sludge), municipal wastewater sludges include sludges from mechanical pre-settling (primary sludge) and chemical phosphorus precipitation. Sludge from the phosphorus precipitation can be referred to as primary, secondary or tertiary sludge depending on the precipitation method (discussed earlier in Chapter 3). (Metcalf & Eddy, Inc. 2003; Karttunen et al. 2004; Finland's Environmental Administration 2011.)

Sludges produced by different treatment methods and dry matter contents are presented in Table 4.2. In total, approximately 0.7 - 1.2 kg dry matter is produced per 1 kg of removed BOD_7. Treatment of excess sludge may constitute approximately one third of the WWTP's operating costs. (Karttunen et al. 2004; Finland's Environmental Administration 2011.)

Table 4.2. *Sludges produced by different treatment methods and dry matter contents (Karttunen et al. 2004).*

Treatment method		Sludge dry solids		Sludge	Dry matter
		[g/ m^3 wastewater]	[g/inhab.*d]	[l/inhab.*d]	[%]
1. Mechanical		150	60	1 - 2	3 - 6
2. Biological					
- act. sludge, pre-settling		90	35	1 - 3	0.5 - 3
- act. sludge, no pre-settling		240	95	2.5 - 1.5	1 - 4
- extended aeration		160	65	1.5 - 3	1 - 4
3. Simultaneous precipitation					
- act. sludge		320	130	2.5 - 5	1.5 - 5
- extended aeration		240	100	2 - 4	1 - 4
4. Chemical precipitation					
- chem. settling	$Ca(OH)_2$ 300 g/m^3	600	240	2.5 - 6	4 - 10
- direct precipitation	$Al(SO_4)_3$ 125 g/m^3	70	30	1 - 3	1 - 3
	$FeCl_3$ 70 g/m3	100	45	1 - 3	1.5 - 4.5
- after precipitation	$AL_2(SO_4)_3$ 125 g/m^3	60	25	1 - 2.5	1 - 2

Sludges from different processes have their own typical characteristics. Primary sludge contains the settleable fraction of the wastewater, such as fibres, food wastes, sand and excrements. Primary sludge is easily decomposable, relatively concentrated and viscous, and has poor fluidity due to undegradable slimy organic colloids. Secondary sludge consists mainly of dead microbes. It is relatively watery and does not contain slimy viscous substances. Thus, secondary sludge has good fluidity. Sludge from chemical precipitation typically consists of phosphate, hydroxide and carbonate deposits. Typical chemical compositions of untreated primary and activated sludge are presented in Table 4.3. Due to relatively high added chemical concentrations, the characteristics of chemical precipitation sludge are determined by the used chemicals. (Metcalf & Eddy, Inc. 2003; Karttunen et al. 2004; Finland's Environmental Administration 2011.)

Table 4.3. *Typical chemical composition of untreated primary and activated sludge (Metcalf & Eddy, Inc. 2003; Karttunen et al. 2004).*

Item	Untreated primary sludge		Untreated activated sludge
	Range	Typical value	Range
Total dry solids [TS, %]	5 - 9	6	0.8 - 1.2
Volatile solids [% of TS]	60 - 80	65	59 - 88
Grease and fats [% of TS]			
- ether soluble	6 - 30	n.i.a.	n.i.a.
- ether extract	7 - 35	n.i.a.	5 - 12
Protein [% of TS]	20 - 30	25	32 - 41
Nitrogen [N, % of TS]	1.5 - 4	2.5	2.4 - 5.0
Phosphorus [P_2O_5, % of TS]	0.8 - 2.8	1.6	2.8 - 11
Potash, [K_2O, % of TS]	0 - 1	0.4	0.5 - 0.7
Cellulose [% of TS]	8 - 15	10	n.i.a.
Iron [not as sulphide, % of TS]	2 - 4	2.5	10 - 20*
Silica [SiO_2, % of TS]	15 - 20	n.i.a.	n.i.a.
pH	5 - 8	6	6.5 - 8
Alkalinity [$CaCO_3$, mg/L]	500 - 1 500	600	580 - 1 100
Organic acids [HAc, mg/L]	200 - 2 000	500	1 100 - 1 700
Energy content [kJ/kg TS]	23 000 - 29 000	25 000	19 000 - 23 000

n.i.a. = no information available

* simultaneous precipitation

Usually all sludge fractions are lead to the same excess sludge treatment, as presented earlier in Figure 2.3. Also sludges from the cesspools and small scale treatment plants are delivered to WWTPs, mixed with incoming wastewater or with WWTPs' own sludges. (Metcalf & Eddy, Inc. 2003; Karttunen et al. 2004; Finland's Environmental Administration 2011.)

4.3 Excess Sludge Processing and Utilization

In addition to organic material and nutrients, excess sludge from municipal wastewater treatment contains the same large variety of microorganisms than activated sludge, constituting a major health risk if not processed properly. Decomposing sludge also produces large volumes of methane gas and odour disadvantages. Thus, the main goals in sludge processing have been decreasing the sludge volume by removing water, stabilizing the sludge in order to stop the decomposition and making sludge hygienically safe. Other excess sludge processing methods aim to contribute the further utilization, transportation and final disposal of the excess sludge. The main sludge processing methods and their orders are presented in Figure 4.3. (Karttunen et al. 2004; Santala & Etelämäki 2009; Finland's Environmental Administration 2011.)

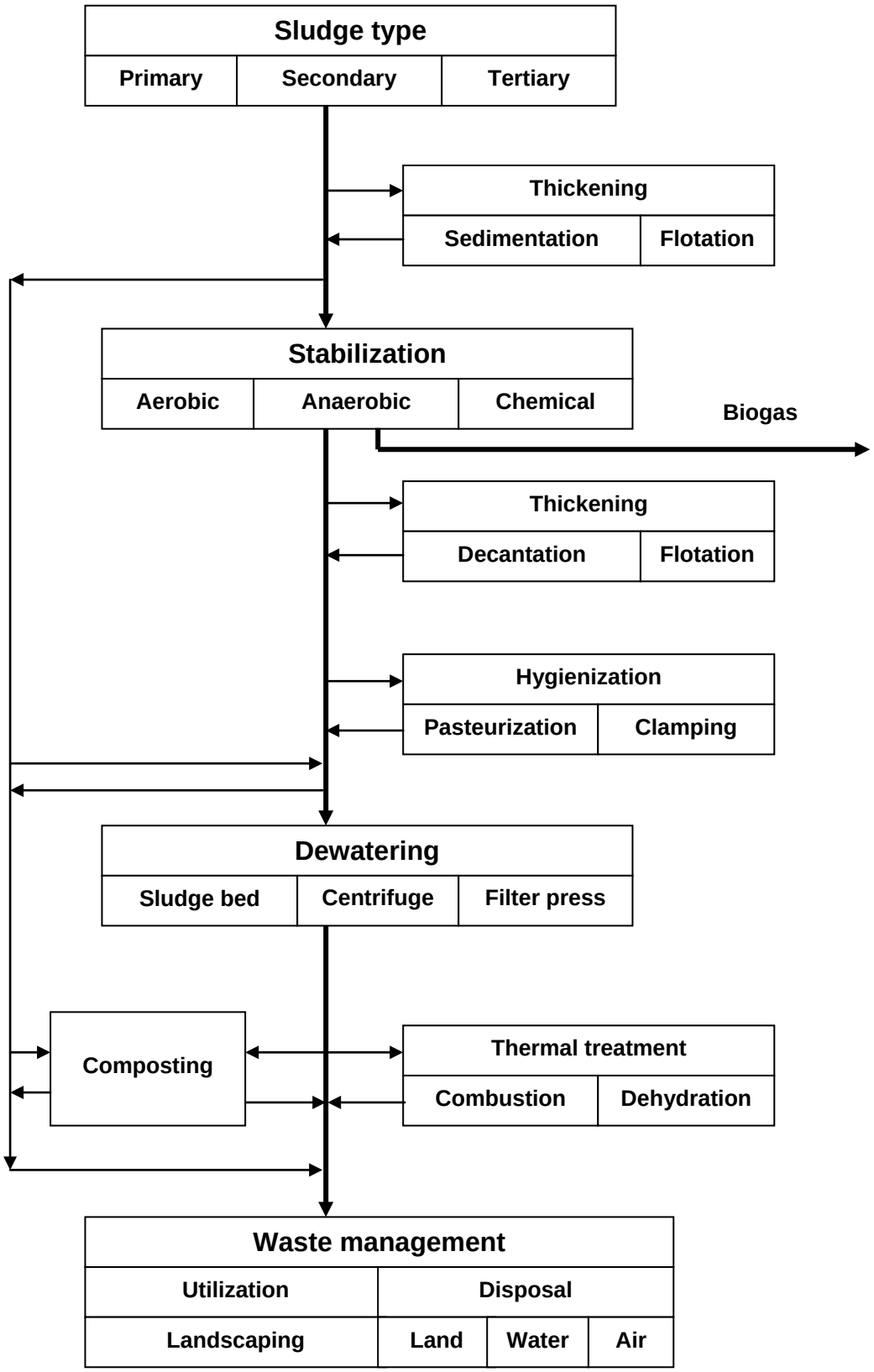

Figure 4.3. *The main sludge processing methods and their order (Karttunen et al. 2004).*

As seen from the Figure 4.3, excess sludge processing includes several biological, mechanical and chemical methods. Thickening the excess activated sludge is usually done by adding polymeric chemical, for example at Viinikanlahti WWTP approximately 5 mg of Kemira Fennopol is added per 1 L of sludge. With polymer addition approximately 2-3 times higher dry solid concentration is achieved. After decantation, the separated water is returned into the wastewater treatment. The organic sludge matter can be degraded to a great extent using biological methods, anaerobic and aerobic digestion alike. Especially anaerobic digestion reduces the concentration of active microorganisms in the sludge. Also stabilization with lime is used on some WWTPs. The most common method for processing the stabilized sludge is dewatering using centrifuge, filter press or other corresponding device. After dewatering, the sludge is usually composted. In addition to composting, thermal drying or combustion of the sludge are used in certain extent. Also direct composting after sedimentation is used, especially in smaller WWTPs. (Karttunen et al. 2004; Santala & Etelämäki 2009; Sandelin 2010; Finland's Environmental Administration 2011.)

4.3.1 Energy Production from Excess Sludge

Anaerobic digestion is among the oldest processes for the stabilization of biosolids such as excess sludge. In addition to less microbiologically active sludge, anaerobic digestion produces sufficient biogas to be used either for heat or electricity production at the WWTP, or even to be refined and sold as a traffic propellant. Anaerobic digestion is a three stage process, which takes place in a special digestion tank in the absence of molecular oxygen. In the first step, particulate matter is converted to soluble compounds that can be hydrolyzed further to simple monomers (e.g. lipids to fatty acids) that are used by bacteria that perform the second stage, fermentation. In the fermentation (a.k.a. acidogenesis), organic substrates are degraded further, and final products are acetate, hydrogen and carbon dioxide (CO_2). The final stage is methanogenesis, in which methane (CH_4) is produced by the group of Archaea, *methanogens*: acetate fermenters split acetate into CH_4 and CO_2, while hydrogen oxidizers use CO_2 as a carbon source and electron acceptor, and hydrogen (H_2) as an electron donor, producing CH_4. Sulphur compounds are reduced into hydrogen sulphide (H_2S) and nitrogen compounds into ammonia (NH_3). If process temperature is mesophilic (approx. 30 - 37 ^oC), sludge remains in the tank for 15 - 30 days. Under thermophilic conditions (approx. 50 - 55 ^oC), process takes less time, larger fraction of the sludge is digested and the end product is more hygienic. During the digestion, pathogens and other detrimental organisms such as worm eggs are deceased, and 50 - 70% of organic matter degrades to biogas. Biogas of a good quality contains 65 - 70% CH_4 and 25 - 30% CO_2, the rest is H_2S, NH_3 and other impurities. Approximately 1 - 1.1 m^3 of biogas is produced per 1 kg of digest sludge. Excess sludge can also be digested together with the organic fraction of municipal solid wastes. (Rittmann & McCarty 2001; Sosnowski et al. 2003; Metcalf & Eddy, Inc. 2003; Finland's Environmental Administration 2011.)

Also combustion of the sludge may serve as sludge utilization method, if the sludge can be dried energy efficiently. Advantages of combustion are utilization of all the sludge's energy content, destruction of pathogens and decreased sludge volume. Disadvantages include high energy, operation and maintenance costs as well as environmentally adverse emissions and residuals (e.g. nitrogen oxides, dioxins, furans, heavy metals). Sludges may be combusted separately or in combination with municipal solid wastes. It is unnecessary or even detrimental to stabilize sludge before combustion, because especially digestion decreases the volatile content of the sludge, leading to increased requirement for auxiliary fuel. There are various methods for sewage sludge combustion, from which multiple hearth and fluidized bed furnaces are the most popular. (Johnson 1994; Hein & Bemtgen 1998; Werther & Ogada 1999; Metcalf & Eddy, Inc. 2003; Finland's Environmental Administration 2011.)

4.3.2 Excess Sludge Utilization and Disposal

Formerly the excess sludge was widely used in agriculture as a fertilizer or as a backfill. However, combining the municipal and industrial wastewaters may make the utilization of the sludge more difficult because of the harmful substances, e.g. heavy metals, in industrial waters. In EU, sludge disposal is licensed and limited by legislations. (LeBlanc et al. 2008; Muga & Mihelcic 2008; Rantanen et al. 2008; Santala & Etelämäki 2009; Finland's Environmental Administration 2011.) Table 4.4 presents the differences between some European countries on sludge utilization and disposal, as well as differences in documentation exactness.

Table 4.4. *Excess sludge utilization and disposal in Europe (Rantanen et al. 2008).*

Country	Sludge utilization and disposal
UK	65% for agriculture, compost or landscaping, 35% to landfill or combusted (incl. cement fabrication)
Germany	for agricultural use or combustion
Greece	100% disposed to landfill sites
Norway	50% for agricultural use, 50% for landscaping
France	60% for landscaping and agricultural use, 17% combusted, 20% to landfill
Holland	47% combusted, 34% thermally dried for cement fabrication, 14% for landscaping, 5% exported for combustion
Italy	landscaping, composting, decomposition and landfill disposal
Belgium	45% dried and combusted, 29% combusted, 14% for landscaping, 12% for agricultural use
Austria	for agriculture and landscaping, dried and composted, combusted, to landfill for methane production
Estonia	40% for landscaping, 45% for agricultural use, 10% for gardening, 5% to landfill
Finland	80% for landscaping, 12% for agricultural use, 6% to landfill

As seen from Table 4.4, the most usual sludge utilization methods in Europe are landscaping and agricultural use. Combustion is usual, while disposal in landfill sites is rela-

tively rare except in Greece. Sludge is also utilized in cement fabrication in UK and Holland. In Austria, sludge is used as a methane oxygenation layer at the landfill sites to enhance methane production. (Rantanen et al. 2008.)

4.4 Excess Sludge as Biodiesel Feedstock

As mentioned before, it has been noted that the lipid content of excess sludge could be utilized as a feedstock for biodiesel, offering a new non food-related biodiesel source. The lipid fraction of the municipal wastewater sludge is a composite organic matrix originating from the direct adsorption of lipids from domestic and industrial wastes to the sludge, and/or from the phospholipids in the cell membranes of microorganisms, their metabolites and by-products of cell lysis. By far, the most common method for biodiesel production is transesterification, a process where bacterial; animal or plant fats are reacted with alcohol (usually methanol) to give fatty acid methyl esters (FAME, a.k.a. biodiesel) and glycerol. Acid or alkali catalyst is used to help the reaction proceed faster. (Knothe et al. 2005; Dufreche et al. 2007; Angerbauer et al. 2008; Mondala et al. 2009; Revellame et al. 2009; Pokoo-Aikins et al. 2010).

Dufreche et al. (2007) compared biodiesel yields from municipal secondary sludge originating Tuscaloosa WWTP, USA using different extraction procedures. They tested accelerated solvent extraction (ASE®) using different organic solvents, supercritical CO_2 extraction, and *in situ* transesterification. A total lipid content of 27.43 mass-% (g lipids/g dried sludge) was achieved using a mixture of hexane, methane and acetone. The *in situ* transesterification extraction procedure gave the highest yield of biodiesel, 6.23 mass-% (g FAMEs/g dried sludge), since the reagents have access to all lipids in the feedstock. However, Dufreche et al. (2007) further stated that with an assumed FAME yield of 10 mass-%, outfitting 50% of all existing municipal wastewater treatment plants in the USA for lipid extraction and transesterification, biodiesel could be produced to meet 0.5% of the annual petroleum diesel demand in the USA ($0.7 * 10^6$ m^3).

Mondala et al. (2009) studied the FAME yields and fatty acid compositions of primary and secondary sludges from the same Tuscaloosa WWTP that Dufreche et al. (2007). Transesterification method with different temperatures, acid catalyst concentrations, and methanol to sludge mass ratios were compared. Gas chromatography (GC) analysis of the FAMEs from both types of sludge indicated the occurrence of significant concentrations of the methyl esters of palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1), which are suitable for biodiesel production. The maximum mass-% FAME yields were obtained at 75 ^oC, 5% (v/v) H_2SO_4, and 12:1 methanol to sludge mass ratio and were 14.5% and 2.5% for primary and secondary sludge, respectively. An economic analysis estimated the cost of 1.65 €/L ($3.23/gallon) for a neat biodiesel obtained from this process at an assumed yield of 10% FAMEs/dry weight of sludge.

Based on previous results, to make sewage sludge competitive with conventional oil crops as biodiesel feedstock in terms of biodiesel yield, its lipid content must be in-

creased (Mondala et al. 2011). Many organisms synthesize lipids as an integral part of their metabolism and as energy and carbon storage compounds needed for maintenance of metabolism and synthesis of cellular metabolites during starvation and in particular if growth resumes. Among eukaryotic organisms such as yeasts, fungi, plants and animals, biosynthesis of triacylglycerides (TAGs) is very common. Prokaryotes are also able to accumulate lipophilic storage compounds, although mainly specialized lipids such as poly(3-hydroxybutyric acid) or other polyhydroxyalkanoic acids. However, it has been discovered that also TAG accumulation is widespread among bacteria. (Alvarez & Steinbüchel 2002; Wältermann et al. 2005; Wältermann & Steinbüchel 2005).

Oleaginous microorganisms are referred to as microbial organisms that can accumulate intracellular lipids over 20% of their dry biomass weight. Even 70% lipid contents have been reported in response to high carbon and low nitrogen concentration (i.e. high C/N ratio). One suggested possibility is to design new biological WWTPs, where oleaginous microorganisms would be grown on municipal wastewater. In addition to wastewater treatment, the excess oleaginous biomass could be used as lipid feedstock. Although various kinds of microorganisms, such as microalgae, bacteria, fungi and yeast, are able to storage oils, not all of them are suitable for biodiesel production. The success of the process depends heavily on the performance of the oleaginous consortium, and whether or not it is able to compete for carbon and other nutrients with the indigenous microorganisms contained in the wastewater. (Ratledge & Wynn 2002; Meng et al. 2009; Zhou et al. 2011; Hall et al. 2011.)

One possibility is to combine conventional biological wastewater treatment and enhanced production of lipid rich sludge for biodiesel feedstock. More intracellular lipids are known to be accumulated in response to high C/N ratio, and activated sludge is known to contain various microbial populations, even oleaginous. (Ratledge & Wynn 2002; Rittmann & McCarty 2001; Mondala et al. 2011.) Thus, in a study by Mondala et al. (2011), enhanced accumulation of biofuel feedstock lipids and FAMEs in activated sludge was attempted by manipulating the C/N ratio and glucose loading in batch cultivation activated sludge bioreactors. Also lipid and biomass accumulation kinetics were studied. At high initial C/N ratio (≥40:1) and glucose loading (≥40 g/L), higher content (mass-% of dried sludge) of lipids and FAMEs was achieved, compared to raw activated sludge (RAS). With a C/N ratio of 70:1 and glucose loading of 60 g/L, a maximum of 17.5 ± 3.9 mass-% lipid content with a corresponding biodiesel yield to 10.2 ± 2.0 mass-% was achieved after 7 days of cultivation. RAS had average lipid content of 11.0 ± 1.7 mass-% (2.84 ± 0.45 mass-% biodiesel). Volumetric lipid yields, however, were higher at C/N ratio of 40:1 than 70:1, approximately 2 250 mg/L and 1 400 mg/L respectively, due to a higher total biomass yield in the former.

Additionally, in Mondala's et al. (2011) study, the resulting fatty acid profiles indicated that lipids derived from activated sludge grown aerobically in glucose-containing synthetic wastewater medium are suitable for the production of biodiesel with improved cold flow, ignition property (cetane number), and oxidative stability. Microbial composition in activated sludge exposed to C/N ratio of 70:1 shifted towards specific gammap-

roteobacteria, suggesting their relevance in lipid production in wastewater microbiota and potential value in biofuel synthesis applications.

Mondala et al. (2011) also published their proposition of a WWTP configuration that utilizes the microbial community in municipal activated sludge in an aerobic fermentation process (Figure 4.4).

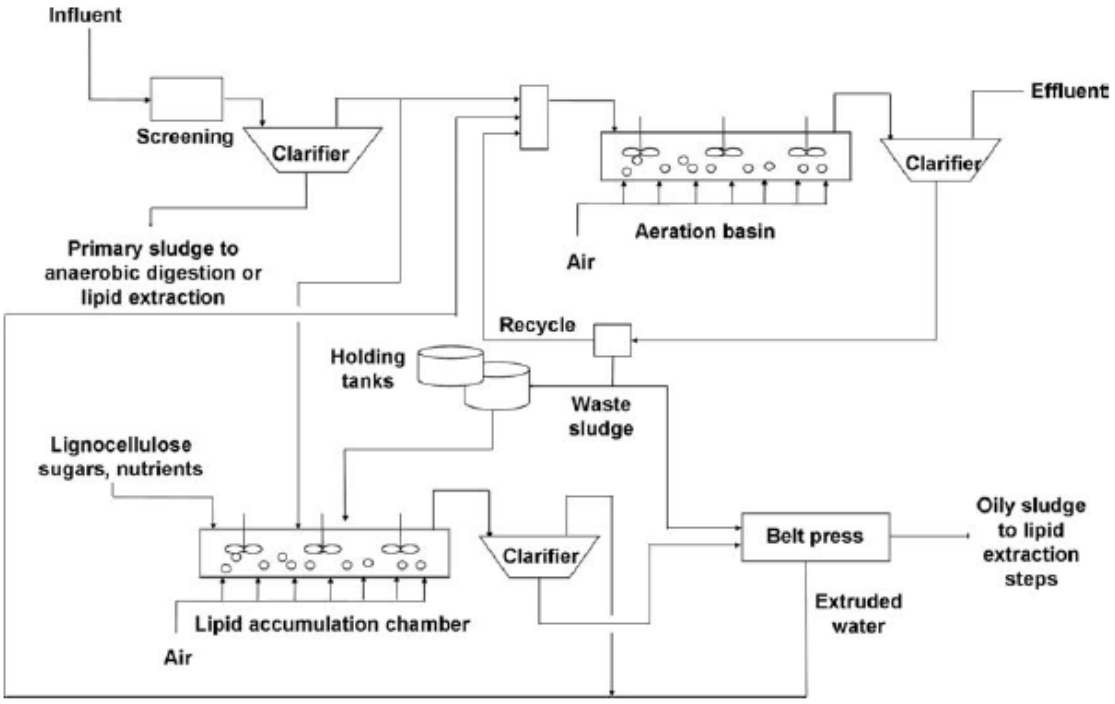

Figure 4.4. *Proposed WWTP concept for the enhancement of lipid and biodiesel yields from activated sludge (Mondala et al. 2011).*

In a proposition by Mondala et al. (2011), the waste excess sludge is directed into lipid accumulation chamber. External sugar or other carbon source and (possibly) nutrients are added to enhance the accumulation of storage lipids. Oily sludge is recovered for lipid extraction. Extruded water from the process is returned into the aeration basin for proper treatment.

5 WASTEWATER TREATMENT AND EXCESS SLUDGE PRODUCTION IN FINLAND

In 2007, 83% of Finland's population lived in urbanized areas (distance between buildings less than 200 meters, over 200 inhabitants). Based on the calculations on the population, sewer network connections and the measured total organic loading of the WWTPs, it can be stated that all urbanized areas in Finland are connected to the wastewater treatment. In 2007, the total wastewater inflow to WWTPs in Finland was approximately 500 Mm^3, and total wastewater sludge production was approximately 150 000 tons in dry weight. The number of WWTPs treating wastewaters of more than fifty inhabitants was 540. On average 97, 96 and 56% of organic matter (BOD_{7ATU}), phosphorus and nitrogen was removed, respectively. (Santala & Etelämäki 2009.)

Approximately 35% of Finland's WWTPs in 2007 involved biological treatment, phosphorus and nitrogen removal. Rest of the WWTPs (approx. 65%) involved biological treatment and phosphorus removal. New WWTPs are usually designed for more effective nitrogen removal using techniques discussed earlier in Chapter 3. Towards year 2015, it is estimated that the wastewater organic load, as well as nutrient load will increase from 15 to 20%. New legislations on the wastewater treatment of sparsely populated areas may also have significant effects on some WWTPs, when sludges from the cesspools and small scale treatment plants are delivered to WWTPs for after-treatment. Usually the delivered sludges are processed together with sludges formed in the WWTP. (Santala & Etelämäki 2009.)

Data on wastewater treatment, sludge production and utilization in Finland's cities with more than 50 000 inhabitants is presented in Table 5.1, Table 5.3, Table 5.4, Table 5.5 and Table 5.6. The data is based on the Information System of Finland's Environmental Administration (Vahti 2010), WWTP's environmental licenses (Finland's Environmental Administration 2011), WWTPs internet pages and annual reports. The 19 presented WWTPs treat more than half of Finland's treated wastewaters (284 Mm^3 in 2010) and produce approximately 69 000 tons of dried wastewater sludges annually. Volume of industrial wastewaters treated varies widely between WWTPs. The most widely used wastewater treatment technique is activated sludge process with simultaneous chemical precipitation.

The four largest WWTPs in Finland are Viikinmäki in Helsinki, Suomenoja in Espoo, Kakolanmäki in Turku and Viinikanlahti in Tampere. Those WWTPs process approximately 35% of Finland's treated wastewaters and produce approximately 26% of the dried wastewater sludges. Some fundamental process parameters of the four largest WWTPs are presented in Table 5.2.

Table 5.1. *Largest WWTPs in Finland, processes and wastewater inflow (Vahti 2010)*

City	WWTP	Process	Overall wastewater inflow		Industrial wastewaters	
			Inf. [m^3/a]	Inf. BOD_{7ATU} [kgO_2/a]	[% of total Inflow]	[% of total BOD_{7ATU}]
Helsinki &Vantaa	Viikinmäki	AS/SP /DN/BF	97 612 003	21 587 734	2	4
Espoo	Suomenoja	Pre-DN /AS/SP	32 882 571	7 139 788	8	n.i.a.
Turku	Kako-lanmäki	AS/SP /DN/AF	28 507 000	8 586 000	n.i.a.	n.i.a.
Tampere	Viinikanlahti	AS/SP	18 278 784	3 796 760	15	20
Oulu	Taskila	AS/SP /DN/BF	15 100 000	3 816 075	n.i.a.	n.i.a.
Jyväskylä	Nenäin-niemi	AS/SP	12 818 692	4 704 231	17	n.i.a.
Pori	Luotsinmäki	AS/SP /DN/AF	8 518 655	3 269 730	n.i.a.	n.i.a.
Lahti	Kariniemi	AS/SP /DN	6 725 639	3 034 306	n.i.a.	n.i.a.
Kuopio	Lehtoniemi	AS/SP	6 697 000	2 756 900	n.i.a.	n.i.a.
Vaasa	Pått	AS/SP	6 507 392	1 520 582	n.i.a.	n.i.a.
Kotka	Mussalo	AS/SP	6 372 566	2 043 234	10	52
Seinäjoki	Central WWTP	AS/SP /DN/*	6 148 908	2 239 570	12	n.i.a.
Joensuu	Kuhasalo	AS/SP /AP	6 056 593	1 385 637	n.i.a.	n.i.a.
Rovaniemi	City WWTP	AS/SP /AF	5 936 042	1 275 080	n.i.a.	n.i.a.
Lap-peenranta	Toikansuo	PP/AS /AP	5 474 244	2 107 404	n.i.a.	n.i.a.
Hämeen-linna	Paroinen	AS/SP/F	5 397 918	1 815 836	n.i.a.	n.i.a.
Kouvola	Mäkikylä	AS/SP	5 382 112	1 175 936	n.i.a.	n.i.a.
Salo	Central WWTP	AS/SP /BF	5 020 680	822 614	n.i.a.	n.i.a.
Lahti	Ali-Juhakkala	AS/SP /DN	4 542 735	2 115 005	n.i.a.	n.i.a.
SUM			**283 979 534**	**75 192 422**	n.i.a.	n.i.a.

AS = Activated Sludge
AF = After-filtration
AP = After-precipitation
BF = Biological Filtration
DN = Denitrification – Nitrification
F = Flotation
PP = Pre-precipitation
SP = Simultaneous Precipitation
n.i.a. = no information available
*Flotation and Biofiltration for Industrial WWs

Table 5.2. *The four largest WWTPs in Finland, process parameters (Finland's Environmental Administration)*

City	BOD_{7ATU}	V_{tot}	$V_{aeration}$	MLSS Aer.	Volumetric Load	F/M ratio	HRT
	[g/m^3]	[m^3]	[m^3]	[g/m^3]	[kg BOD_{7ATU}/ d/m^3 aeration]	[kgBOD_{7ATU}/ kgMLSS*day]	[h]
Helsinki &Vantaa	221	245 450	88 000	4 400	0.45	0.09	22
Espoo	217	96 122	36 000	4 400	0.29	0.07	26
Turku	301	99 365	56 300	3 500	0.42	0.06	31
Tampere	208	57 184	17 000	4 500	0.19	0.04	27

Table 5.3. *Largest WWTPs in Finland, BOD reduction (Vahti 2010)*

City	WWTP	BOD_{7ATU} [kgO_2/a]		BOD reduct. [%]
		Inf.	Eff.	
Helsinki &Vantaa	Viikinmäki	21 587 734	947 967	96
Espoo	Suomenoja	7 139 788	152 854	98
Turku	Kakolanmäki	8 586 000	160 160	98
Tampere	Viinikanlahti	3 796 760	85 304	98
Oulu	Taskila	3 816 075	77 380	98
Jyväskylä	Nenäinniemi	4 704 231	123 843	97
Pori	Luotsinmäki	3 269 730	352 684	89
Lahti	Kariniemi	3 034 306	37 492	99
Kuopio	Lehtoniemi	2 756 900	39 446	99
Vaasa	Pått	1 520 582	73 698	95
Kotka	Mussalo	2 043 234	42 164	98
Seinäjoki	Central WWTP	2 239 570	33 009	99
Joensuu	Kuhasalo	1 385 637	37 487	97
Rovaniemi	City WWTP	1 275 080	49 973	96
Lappeenranta	Toikansuo	2 107 404	43 385	98
Hämeenlinna	Paroinen	1 815 836	18 900	99
Kouvola	Mäkikylä	1 175 936	71 989	94
Salo (2006)	Central WWTP	822 614	25 759	97
Lahti	Ali-Juhakkala	2 115 005	26 214	99
SUM		**75 192 422**	**2 399 708**	
Average				**97**

Inf. = Influent
Eff. = Effluent

Table 5.4. *Largest WWTPs in Finland, nutrient reduction (Vahti 2010)*

City	P_{tot} [kg/a]		P_{tot} reduct. [%]	N_{tot} [kg/a]		N_{tot} reduct. [%]
	Inf.	Eff.		Inf.	Eff.	
Helsinki &Vantaa	673 703	29 429	96	4 550 683	638 509	86
Espoo	283 763	10 900	96	2 069 937	519 863	75
Turku	224 500	5 617	97	1 514 400	329 440	78
Tampere	108 822	5 017	95	740 700	591 320	20
Oulu	120 678	2 326	98	820 360	427 157	48
Jyväskylä	136 510	3 139	98	826 725	410 625	50
Pori	66 525	5 516	92	429 612	210 477	51
Lahti	64 496	1 874	97	373 121	77 438	79
Kuopio	72 090	1 550	98	447 100	286 020	36
Vaasa	50 333	2 901	94	385 479	241 358	37
Kotka	43 518	2 641	94	357 209	78 364	78
Seinäjoki	56 823	2 398	96	313 947	119 837	62
Joensuu	67 008	1 236	98	339 357	315 784	7
Rovaniemi	51 179	1 252	98	343 099	267 888	22
Lappeenranta	64 049	2 115	97	361 149	140 022	61
Hämeenlinna	51 720	1 542	97	308 702	180 764	41
Kouvola	37 303	3 712	90	244 264	166 059	32
Salo (2006)	38 043	2 250	94	202 614	147 412	27
Lahti	49 911	1 150	98	248 165	67 598	73
SUM	**2 260 974**	**86 565**		**14 876 623**	**5 215 935**	
Average			**96**			**51**

Table 5.5. *Largest WWTPs in Finland, sludge processing and production (Finland's Environmental Administration)*

City	Sludge Processing	Dried sludge [tons/a]	Dry matter content [%]	Dry mass [tons/a]
Helsinki &Vantaa	DE-ST/DC/MDR	56 000	29	16 240
Espoo	TP/DE-ST/MDR	25 000	29	7 250
Turku	DE-ST/MDR/TD	34 000	28	9 520
Tampere	TP/DE-ST/MDR	22000 [m3]	30	6 600
Oulu	Kemicond*/MDR	26 000	22	5 720
Jyväskylä	TP/MDR/DE-ST	n.i.a.	32	n.i.a.
Pori	TP/MDR/CO/CST	20 000	20	4 000
Lahti	TP/DE-ST/MDR	13 000	26	3 380
Kuopio	TP/DE-ST/MDR	7 000	26	1 820
Vaasa	TP/MDR/ DE-ST(outsourced)	14 000	15	2 100
Kotka	TP/MDR/CO	7 300	28	2 044
Seinäjoki	TP/MDR/ DE-ST(outsourced)	10 000	24	2 400
Joensuu	TP/DE-ST/MDR/TD	1 700	83	1 411
Rovaniemi	TP/CO	10 000	25	2 500
Lappeenranta	TP/CO	9 000	21	1 890
Hämeenlinna	TP/DE-ST/MDR	6 890	22	1 516
Kouvola	TP/MDR/CO/ (DE-ST in the future)	6 000	11	660
Salo	TP/MDR	n.i.a.	n.i.a.	n.i.a.
SUM		**245 890**		**69 051**
Average			**28**	

DC = Decantation
DE-ST = Decomposition – Stabilization
CST = Chemical Stabilization
CO = Composting
MDR = Mechanical Drying
TD = Thermal Drying
TP = Thickening with Polymer

* Chemical Stabilization and Drying with Acid and Hydrogen Peroxide

Table 5.6. *Largest WWTPs in Finland, sludge utilization (Finland's Environmental Administration)*

City	Utilization	Biogas production [m^3/a]	Methane proportion [%]	Energy from biogas [GWh/a]	Heat from biogas [GWh/a]
Helsinki &Vantaa	BG --> CO --> LS	10 000 000	65	16	26
Espoo	BG --> CO --> LS	3 613 500	63	4.5	6
Turku	BG --> CO --> LS	3 300 000	55-75	6.9	11.4
Tampere	BG --> CO --> LS/AG	1 900 000	60	3	3.5
Oulu (2009)	(BG)* --> CO --> AG	-	-	-	-
Jyväskylä	BG --> CO --> LS/AG	n.i.a.	62-65	n.i.a.	n.i.a.
Pori	LS/AG	-	-	-	-
Lahti	BG --> CO --> LS/AG	2 610 000	55-75	-	15
Kuopio	BG --> CO --> LS	1 200 000	66	2	3.6
Vaasa	BG --> CO --> LS (outsourced)	930 000	65	n.i.a.	n.i.a.
Kotka	Under Design	-	-	-	-
Seinäjoki	BG --> CO --> LS (outsourced)	n.i.a.	n.i.a.	n.i.a.	n.i.a.
Joensuu	BG --> LS	800 000	55-75	1.2	1.4
Rovaniemi	CO --> LS/AG	-	-	-	-
Lap-peenranta	CO --> LS/AG	-	-	-	-
Hämeen-linna	BG --> CO --> LS/AG	n.i.a.	n.i.a.	n.i.a.	n.i.a.
Kouvola	(BG)* --> CO --> AG	n.i.a.	n.i.a.	n.i.a.	n.i.a.
Salo (2006)	n.i.a.	n.i.a.	n.i.a.	n.i.a.	n.i.a.
SUM		**24 353 500**		**34**	**67**
Average			**64**		

AG = Agricultural Use
BG = Biogas
CO = Composting
LS = Landscaping

* In the Future

The excess sludge is usually dried and decomposed, and biogas is utilized as heat and energy. Theoretical biogas production from the sludges of 19 largest WWTPs is approximately 72 Mm^3. The decomposed sludge is further utilized in landscaping or in agricultural use. Total biogas energy production on the four largest WWTPs is approximately 30 GWh/a, and heat production approximately 47 GWh/a. However, aerobic wastewater treatment consumes more energy than can be produced from the biogas. The four biggest WWTPs buy approximately 37.5 GWh of electricity and 1.5 GWh heat annually. (Finland's Environmental Administration 2011.)

6 MATERIALS AND METHODS

6.1 Municipal Wastewater, Activated and Excess Sludge

Municipal wastewater as well as activated and excess sludges used in the study originated from Viinikanlahti WWTP, which is located near the centre of Tampere city, Finland. The wastewater inflow and organic loading, as well as other fundamental parameters of Viinikanlahti WWTP are previously discussed in Chapter 4.

Wastewater was collected with a submersible pump after preliminary mechanical settling unit operation, from an open basin from which the water entered the aeration basins. In Viinikanlahti WWTP, powdered calcium carbonate and ferrisulphate are added in the wastewater after the mechanical settling. The powders are not mixed with water in a separate unit operation, but the final mixing and dissolution occurs in the aeration basin. Thus, the calcium carbonate (alkalinity) and ferrisulphate concentrations may have slightly varied between samplings.

Activated sludge was collected with a bucket directly from aeration basin. Sludge was collected from the surface, but aeration was considered to provide enough mixing to ensure a representative sample. Excess sludge sample was taken through a safety valve after the thickening basin. The excess sludge contained approximately 5 mg/L polymeric chemical (Fennopol, Kemira), which is added to the thickening basin in order to improve the settling.

Fresh wastewater and sludge were collected and used for each batch bottle experiment. Because of the operation of the WWTP, the characteristics of the wastewater and sludges varied to a certain extent between the experiments. In batch bottle experiments, sludge was used as inoculum and wastewater as the substrate and/or medium. The studied characteristics were mainly determined from the bottles, not separately from wastewater and sludge. However, selected characteristics of wastewater and sludges were as given in Table 6.1.

Table 6.1. *Some characteristics of mechanically clarified municipal wastewater, activated and excess sludge. Because of the operation of the WWTP, the characteristics of the wastewater and sludges varied to a certain extent between the experiments.*

Characteristic	Value		
	Wastewater	**Activated sludge**	**Excess sludge**
pH	7.3 [3)]	7.5 [3)]	n.a.
BOD_{7ATU}	120 mg/L [3)]	1 900 mg/L [1)]	n.a.
COD_s	110 mg/L [3)]	n.a.	n.a.
COD_{tot}	220 mg/L [3)]	4 100 mg/L [1)]	36 000 mg/L [1)]
TSS	95 mg/L [2)]	4 500 mg/L [2)]	53 000 mg/L [1)]
Lipids	n.a.	200 mg/L (5.3 mass-%) [3)]	4 500 mg/L (8.5 mass-%) [3)]
N_{tot}	49 mg/L [3)]	240 mg/L [3)]	n.a.
NH_4-N	35 mg/L [2)]	n.a.	n.a.
P_{tot}	2.7 mg/L [2)]	n.a.	n.a.

n.a. = not analyzed

[1)] Calculated according to the analysis from batch bottle experiments

[2)] According to Sandelin (2010), personal communication.

[3)] According to analysis conducted in TUT laboratory.

6.2 Analytical Methods

6.2.1 Total and Volatile Suspended Solids

Total and volatile suspended solid (TSS and VSS) concentrations were determined according to Finnish Standard SFS 3008 (1990). The glass microfibre filters (Whatman GF/A) were prepared for analysis by submerging them in MilliQ-water for several hours. After washing the filters were placed into aluminium foil cups, dried in 105 ºC for 20 hours and then ignited in 550 ºC for two hours. In the analysis, a known volume of each sample was vacuum filtered through a weighed glass microfibre filter. For TSS determination, the filters were dried in 105 ºC for 20 hours, then cooled and weighed. For VSS determination, same filters were ignited in 550 ºC for two hours, cooled and weighed. Duplicate samples were analyzed. The concentrations of TSS and VSS were calculated using Equations 7.1 and 7.2.

$$TSS = \frac{1000 \cdot (m_2 - m_1)}{V} \tag{7.1}$$

$$VSS = \frac{1000 \cdot (m_2 - m_3)}{V} \tag{7.2}$$

where

TSS = total suspended solids (g/L)
VSS = volatile suspended solids (g/L),
m_1 = combined mass of the cup and filter before sample filtration (g),
m_2 = combined mass of the cup, filter and sample after 20 hours in 105 ºC) (g),
m_3 = combined mass of the cup, filter and sample after 2 hours in 550 ºC (g)
V = volume of the sample (mL).

In laboratory scale activated sludge reactor experiments, TSS is also referred to as mixed liquor suspended solids (MLSS). This is done because in literature and professional language MLSS is the most widely used term used for describing the activated sludge solids. (Metcalf & Eddy, Inc. 1991, Rittmann & McCarty 2001.)

6.2.2 Chemical Oxygen Demand

Chemical oxygen demand (COD) was determined using closed tube method and oxidising with dichromate according to Finnish Standard SFS 5504 (1988). Duplicate samples were analyzed. For soluble COD (COD_s) analysis, samples were filtrated trough Life Sciences IC Acrodisc Syringe Filter with 0.45 µm Supor (PES) membrane. Samples were heated for two hours in a closed tube with strong sulphuric acid (H_2SO_4), silver catalyst, and a known volume of potassium dichromate ($K_2Cr_2O_7$). Oxidable material in the sample reduced a certain amount of $K_2Cr_2O_7$, and remaining $K_2Cr_2O_7$ was determined by titrating with Iron (II) solution. COD- value was then calculated from the consumed $K_2Cr_2O_7$, according to Equation 7.3.

$$COD = \frac{8000 \cdot c_{Fe}(V_1 - V_2)}{V_5} \tag{7.3}$$

where

COD = chemical oxygen demand of the sample (mg/L)
c_{Fe} = concentration of iron (III) solution
V_1 = iron (III) solution consumption of a blank sample
V_2 = (III) solution consumption of a sample
V_3 = volume used in the analysis
8000 = conversion coefficient

6.2.3 Biological Oxygen Demand

Biological oxygen demand for seven days (BOD_{7ATU}) was determined according to Finnish Standard SFS 3019 (1979). Duplicate samples were analyzed. When necessary, the samples were diluted and/or inoculated with wastewater according to the standard. Dissolved oxygen concentration (DO) of the sample was measured directly before and after seven days of incubation in dark and at 20 ºC. The BOD was then calculated according to Equation 7.4, with dilution and the effects of inoculation taken into consideration.

$$BOD_{7ATU} = \frac{V_2}{V_1}[(\rho_1 - \rho_2) - (\rho_3 - \rho_4)] + (\rho_3 - \rho_4) \tag{7.4}$$

where

BOD_{7ATU} = biological oxygen demand after 7 days incubation (mg/L) (+allylthiourea)
V_1= original sample volume (mL)
V_2 = diluted sample volume (mL)
ρ_1 = DO in the beginning of the incubation (mg/L)
ρ_2 = DO (mean) after 7 days incubation (mg/L)
ρ_3 = DO of the blank sample (dilution water) in the beginning of the incubation (mg/L)
ρ_4 = DO (mean) of the blank sample (dilution water) after 7 days incubation (mg/L)

For comparison in some cases, the BOD_{7ATU} was determined using WTWs OxiTop® Control BOD Respirometer System. Measurement using OxiTop® is based on pressure measurement in a closed system. Microorganisms in the sample consume the oxygen and form CO_2 which is absorbed by NaOH. This creates a vacuum that can be measured and converted to mg/L BOD value. Sample dilution and measurement range are depended of the sample volume used. The sample volume regulates the volume of oxygen available for a complete BOD. Measurement ranges of up to 4 000 mg/L can be measured using different volumes. Sampling bottles include measuring head caps, from which the measurement data can be directly read with infrared controller. (WTW 2011.) For both BOD measurement methods, allylthiourea (ATU) was added into the samples in order to rule out the oxygen demand caused by nitrification.

6.2.4 Dissolved Oxygen and pH

Dissolved oxygen concentration (DO, mg/L) and pH were measured straight from the batch bottles as well as from laboratory scale reactors aeration tank and influent canister. The equipment used was WTW Oxi 330i meter with WTW CellOX 325 probe for DO and WTW pH 330i meter with WTW SenTix 41 probe for pH.

Vigorous mixing of the batch bottles as well as bubbling air in the aeration tank may have caused errors in DO and pH measurement. In order to keep the results comparable between experiments, the measurement was done in the same manner from the beginning of the study. In batch bottle experiments, the measurement was always done when mixing was on. If mixing was turned off, the DO started to decrease immediately and often went almost to 0 mg/L. In laboratory reactor experiments, the air bubbles were turned off during DO measurement.

6.2.5 Ammonium and Total Nitrogen

Total nitrogen was determined using Lange LatoN LCK 238 Total Nitrogen analysis kit. Ammonium nitrogen (NH_4-N) was determined with a method modified from the Kjeldahl total nitrogen determination (SFS 5505). Duplicate samples were analyzed. In the Kjeldahl method, all organic and inorganic nitrogen is transformed into ammonium sulphate by boiling in sulphuric acid in a presence of a metal catalyst. This stage was skipped in ammonium analysis, and ammonium was simply distilled from the sample into boric acid solution as ammonia gas. In an acidic solution, ammonia consumed hydrogen ions while transforming into ammonium. The boric acid solution contained indicator, and the ammonium ions were titrated with sulphuric acid (H_2SO_4). Consumption of the sulphuric acid was directly proportional to the ammonium ion concentration (Equation 7.5).

$$2NH_3 + H_2SO_4 \leftrightarrow 2NH_4^+ + SO_4^{2-} \quad (7.5)$$

The ammonium ion concentration was calculated according to Equation 7.6

$$x = \frac{(V_1 - V_2) \cdot c \cdot 18.04 \cdot 2 \cdot 1000}{V} \quad (7.6)$$

where

x	=	ammonium concentration of the sample (mg/L)
V_1	=	sulphuric acid consumed in titration (mL)
V_2	=	sulphuric acid consumed in titration of the 0-sample (mL)
c	=	concentration of the sulphuric acid (mol/L)
V	=	sample volume (mL)
18.04	=	molar mass of ammonium (g/mol)
1000	=	conversion coefficient (mg/g)
2	=	conversion coefficient when titrating with dibasic acid

6.2.6 Lipids analysis

Lipids were extracted from biomass by the modified Bligh and Dyer method (Bligh & Dyer 1959). Volume of 50 - 100 mL of original culture was freeze dried and weighed. A known portion (usually 160 mg) of the dried sample was extracted with 5 mL of chloroform, 10 mL of methanol and 4 mL of Phosphate Buffered Saline (PBS) buffer. The cell suspension was mixed well, shaken on an orbital shaker for 2h at 150-200 rpm and stored overnight at 4°C. The suspension was centrifuged at 6000 rpm for 10 min, the supernatant was separated by decantation, and the sediment was extracted again similar to previous extraction. The combined supernatant was mixed with 10 mL of chloroform and 10 mL of water. Chloroform and water-methanol phases were separated by centrifugation at 6000 rpm for 10 min. The lower (chloroform) phase was collected into a weighed glass vial and purged under nitrogen for evaporation of chloroform. The total lipid content from the extraction was determined gravimetrically. Lipid samples were stored at -20°C.

Extracted lipids were fractionated with thin-layer chromatography (TLC) to study the lipid fraction distribution. TLC equipment consisted of a stationary phase (TLC plate) and a mobile phase (solvent mixture). The TLC plates used were 10x20 cm Silica Gel60 F254 plates with 2.5x10 cm concentration zone (Merck, USA), dyed with iodine for visualization. Mobile phase was n-hexane/diethyl ether/acetic acid solution in ratios 80:20:2, respectively. The samples were spotted at the bottom of the TLC plate which was then placed in a closed glass vessel containing a shallow layer of the solvent. The solvent moved up the TLC plate due to capillary action. The strength of interaction with the mobile and stationary phases is different between each lipid fraction, thus every fraction migrated a different distance and fractions could be recognised from standard samples spotted on the TLC plate at the same time. (Stahl 1967; Clogston & Patri 2011.) Quantity of each fraction was determined semi-quantitatively with digital image analysis using image processing program (ImageJ) (Clogston & Patri 2011).

In batch bottle experiment lipid analysis, activated or excess sludge from Viinikanlahti WWTP was used as initial sample for comparison. Because the batch bottle cultivations were diluted (10% sludge of total volume), the same dilution was taken into account in the initial sample lipid results. Different VSS concentrations between the sludge and batch bottles affected the mg/L lipid concentrations. When VSS decreased, fewer lipids were present in the same volume.

6.3 Batch Bottle Experiments with Municipal Wastewater and Activated Sludge

Two batch bottle experiments were conducted with municipal wastewater and activated sludge. In the experiments, the effects of external organic carbon load on the biomass production and sludge composition were studied. In order to simulate higher organic carbon load, the C/N ratio was elevated by adding different concentrations of glucose into the batch bottles. Activated sludge from Viinikanlahti WWTP was used as an inoculum (10% of total volume) and it was cultivated on incoming wastewater from Viinikanlahti**.** Incubations were done in duplicate 1 L Erlenmeyer flasks at 27 °C. Mixing and aeration were generated by an orbital shaker at a rotation rate 150 rpm. Liquid volume in the beginning of the cultivations was 550 mL.

Experimental arrangements of the experiments with municipal wastewater and activated sludge were as presented in Table 6.2. In the first glucose addition experiment, the biomass production was monitored daily for five days by analysing TSS and VSS.

In the second glucose addition experiment, the length of the cultivation was depending on the concentration of available substrate in the batch bottle. Cultivation continued until the substrate (glucose) ran out, according to COD_s analysis.

Table 6.2. *Initial glucose concentrations, sampling and analysis of the experiments with municipal wastewater and activated sludge. Duplicate samples were analyzed from each bottle.*

Experiment	Initial glucose concentrations [g/L]	Duration [d]	Sampling		
			Daily	Start	End
1st Exp., Initial Glucose Addition	0, 2, 4, 6, 8, 10	5	TSS/VSS pH DO	BOD_{7ATU} COD_{tot} COD_s	BOD_{7ATU} COD_{tot} COD_s
2nd Exp., Initial Glucose Addition	0, 2, 4, 6, 8, 10	1 - 9	COD_s pH DO	TSS/VSS COD_{tot} N_{tot}	TSS/VSS COD_{tot} Lipids

6.4 Batch Bottle Experiments with Municipal Wastewater and Excess Sludge

As seen in Table 6.1, excess sludge from Viinikanlahti WWTP has several different characteristics from activated sludge. When utilization of WWTP sludge is desired without changing the original WWTP process, the most practical solution is to utilise the excess sludge that no longer has a role in the wastewater treatment process. Therefore, excess sludge was used in further experiments.

Experimental arrangements of the three batch bottle experiments with municipal wastewater and excess sludge were as presented in Table 6.3. Effects of higher organic carbon load, different mixing rate, and low temperature on the biomass production and sludge composition were studied. In order to simulate higher organic carbon load, the C/N ratio was elevated by adding different concentrations of glucose into the batch bottles.

Excess sludge from Viinikanlahti WWTP was used as an inoculum (10% of total volume) and it was cultivated on incoming wastewater from Viinikanlahti. Cultivations were done in duplicate 1 L Erlenmeyer flasks at 27 °C or 5.6 °C. Mixing and aeration were generated by an orbital shaker at rotation rates 150 or 300 rpm. Liquid volume in the beginning of the cultivations was 550 mL. In the end of the experiments, lipid mass percentage and lipid distribution of the sludge were analyzed. In the first two experiments, cultivation continued until the substrate (glucose) ran out, according to COD_s analysis. The third excess sludge experiment was conducted at 5.6 ^{o}C, and cultivation was continued 4-6 days after the added glucose (COD_s) had been consumed in order to study the degradation of biomass and lipids when no external organic substrate is available.

Table 6.3. *Experiments with municipal wastewater and excess sludge. Duplicate samples were analyzed from each bottle.*

Experiment	Temperature [^{o}C]	Shaker speed [rpm]	Initial glucose concentrations [g/L]	Spiking	Duration [d]	Sampling			
						Daily	Start	End	½ glucose consumed
Initial Glucose Addition	27	150	0, 2, 4, 6, 8, 10	-	4 - 6	COD_s pH DO	TSS/VSS COD_{tot} N_{tot}	TSS/VSS COD_{tot} Lipids	
Initial Glucose Addition, 300 rpm Mixing Rate	27	150 (all) 300 (8 and 10 g/L glucose)	0, 2, 4, 6, 8, 10	-	3 - 12	COD_s pH DO	TSS/VSS COD_{tot} N_{tot}	TSS/VSS COD_{tot} Lipids	
Initial Glucose Addition, Low Temperature	5.6	300	0, 4, 10	-	17 - 25	COD_s pH DO	TSS/VSS	TSS/VSS* Lipids*	

* When added glucose (COD_s) had been consumed and 4-6 days after

6.5 Laboratory Scale Activated Sludge Reactor

Two experimental runs were conducted with the laboratory scale activated sludge reactor. One objective of the reactor experiments was to study the reactor equipments functions and maintainability, and gain useful insight for future studies. The other target was to study sludge production and treatment efficiency under high organic loads.

Reactors schematic model and set up in the laboratory were as presented in Figure 6.1. The laboratory set up included a control unit, a feed tank, an oxidation tank and a sedimentation tank, sludge removal pump and a scraper motor. The control unit included pumps for influent wastewater and recycle sludge, pump controls, and a flow meter for air. The air was bubbled into the aeration tank via oxygenation block. Air flow rate of 3 L/min was used in order to meet the oxygen requirement and provide efficient mixing. Effluent wastewater was carried to the sewer from the surface of the sedimentation tank.

The temperature in the laboratory varied approximately between 20 and 24 ^{o}C during the experiments. Wastewater and the seed sludge for reactor were brought from Viinikanlahti WWTP. Wastewater was brought twice a week and it was stored in 20 or 30 litre canisters. During the 1st reactor run, the influent canisters were stored first in the cold room at 4 ^{o}C, and brought into the lab before use. In the 1st run, 18.6 L/d wastewater was pumped into the reactor (15.6 L/d in the 2nd run). It was soon discovered that degradation and settling occurred in the influent canisters while the wastewater was being pumped into the reactor. Thus, wastewater canisters had to be stored in the refrigerator at 4 ^{o}C, from which the influent was pumped into reactor via rubber tube.

The reactors operating parameters and other experimental arrangements of the reactor runs were as presented in Table 6.4 and Table 6.5. In the beginning of the first and the second reactor runs, the goal was to scale the reactor parameters to approximately match the ones in Viinikanlahti WWTP. In order to maintain alkalinity and preferred pH, calcium oxide (CaO) had to be added into the influent canister. During the second reactor run the sludge settling problem was partially solved by removing the excess sludge from the aeration tank by a pump. Due to pump modification, the minimum sludge removal rate achieved was 750 mL/d, which caused significantly lower SRT than in Viinikanlahti; 15.2 days compared to approximately 30 days.

Higher organic loads were simulated by adding glucose into the influent. The load was increased in three steps: the COD_s concentration was set up 2, 3, and finally 4 times higher than the initial COD_s concentration of the incoming wastewater. The first step continued for two weeks and the two other steps for approximately one week in order to let the reactor achieve steady state.

Table 6.4. *The laboratory scale activated sludge reactors operating parameters*

Parameter	Unit	1st Run	2nd Run	Viinikanlahti WWTP
Influent BOD_{7ATU}	**[mg/L]**	120 [2)]	120 [2)]	63 [1)]
Initial MLSS	**[mg/L]**	6 100 [2)]	5 000 [2)]	4 500 [1)]
Aeration Tank	**[L]**	7	6	$17 * 10^6$
F/M ratio	**[$kgBOD_{7ATU}$/ kgMLSS*day]**	0.06 [2)]	0.06 [2)]	0.04
HRT	**[d]**	1.5	1.4	1.3
Q_{inf}	**[L/d]**	18.6	15.6	$45*10^6$
$Q_{recycle}$	**[L/d]**	29.0	25.6	$70*10^6$
Recycle Ratio		0.64	0.61	0.61
SRT	**[d]**	-	15.2	30
Air Flow	**[L/min]**	3.0	3.0	Unknown

1) In the beginning of the run, According to Sandelin (2010), personal communication.
2) Initial value, according to analysis conducted in TUT laboratory.

Table 6.5. *Experimental arrangements of the laboratory scale reactor runs*

	Unit	1st Run	2nd Run
Added CaO concentration	**[mg/L]**	0	400
Duration of the run	**[d]**	20	87
1st Glucose Addition (on day 59)	**COD_s [mg/L]**	-	120
2nd Glucose Addition (on day 73)	**COD_s [mg/L]**	-	240
3rd Glucose Addition (on day 78)	**COD_s [mg/L]**	-	360
Control analysis (3 - 5 times a week)		MLSS, COD_s, COD_{tot}, pH, DO (inf. & eff.)	
Control analysis on chosen points		BOD_{7ATU}, NH_4-N (inf. & eff.) Lipids (2nd Run)	

inf. = influent
eff. = effluent

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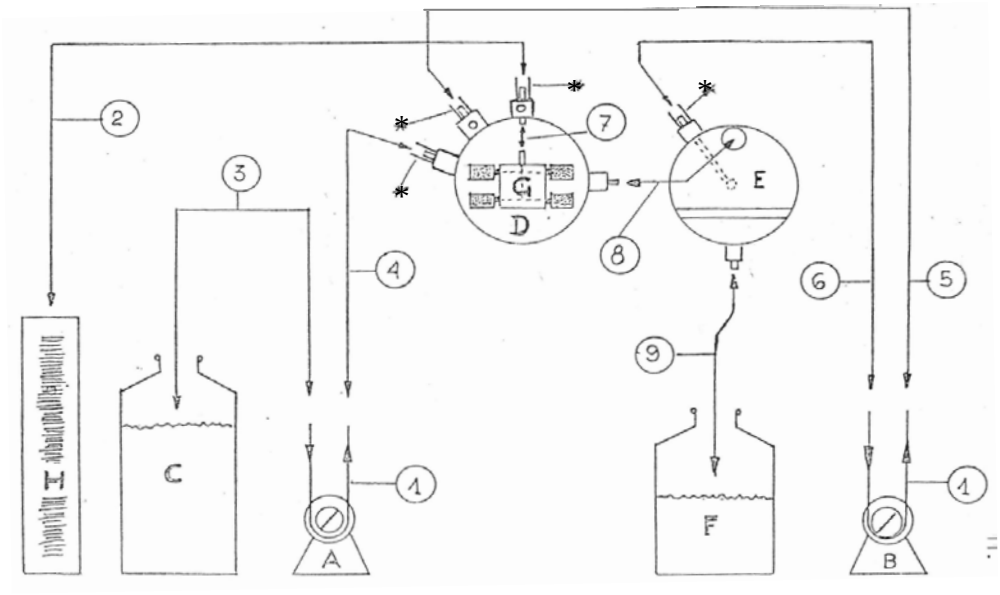

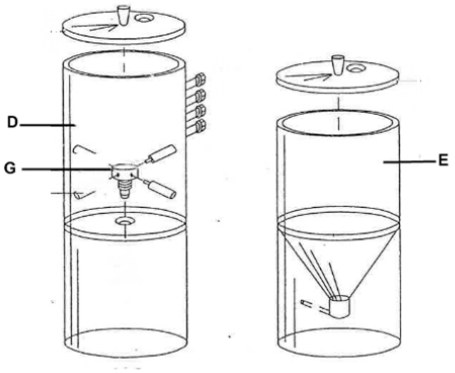

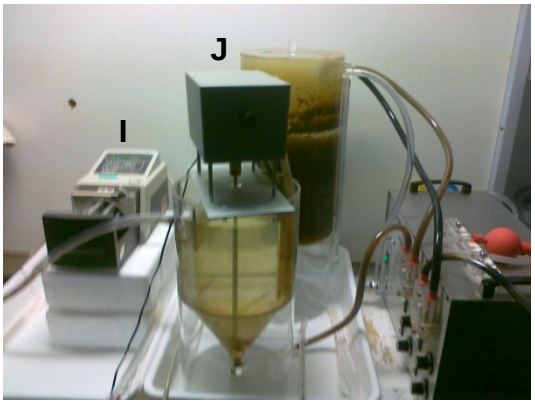

Legend	Volume [L]	Connecting Tubes
A = Feed pump		1 = silicone rubber tube
B = Recycling pump		2 = transparent rubber tube
C = Feed tank	20 - 30	3 = “
D = Oxidation tank	6 - 9	4 = “
E = Sedimentation tank	5.4	5 = “
F = Collecting tank	Sewer	6 = “
G = Oxygenation block		7 = “
H = Pump Control Unit and Flow meter		8 = “
I = Sludge removal pump		9 = “
J = Scraper motor		
* = Connecting tubes		

Figure 6.1. *Laboratory scale activated sludge reactors schematic model and a photograph from the laboratory set up.*

7 RESULTS

7.1 Batch Bottle Experiments with Municipal Wastewater and Activated Sludge

Two batch bottle experiments were conducted with municipal wastewater and activated sludge. In the experiments, the effects of different external organic carbon loads on the biomass production and sludge composition were studied. In order to simulate higher organic carbon load, the C/N ratio was elevated by adding different concentrations of glucose into the batch bottles.

In the first glucose addition experiment, the biomass production was monitored daily for five days by analysing VSS. In the second glucose addition experiment, the length of the cultivation was depending on the concentration of available substrate in the batch bottle. Cultivation continued until the substrate (glucose) ran out, according to COD_s analysis. Error bars in the figures represent standard deviations of the analysis results from parallel batch bottles.

7.1.1 Activated Sludge, the 1st Initial Glucose Addition Experiment

Results from the 1st initial glucose addition experiment were as presented in Figure 7.1, Figure 7.2, Figure 7.3 and Table 7.1. The average initial VSS concentration was 500 ± 70 mg/L. The maximum average biomass increase (in VSS) was from 560 ± 30 mg/L to 1600 ± 70 mg/L, in the batch bottles with initial glucose concentration of 10 g/L. In the bottles where glucose was not added, the VSS concentration decreased approximately from 400 mg/L to 300 mg/L. In the batch bottles with initial glucose concentrations from 4 g/L to 10 g/L, the biomass concentrations still showed increase on the last day of the experiment. Because of the quite large divergence in the VSS results, in the next experiments COD_s was chosen to be analyzed daily in order to find out the end of the logarithmic growth phase for each bottle.

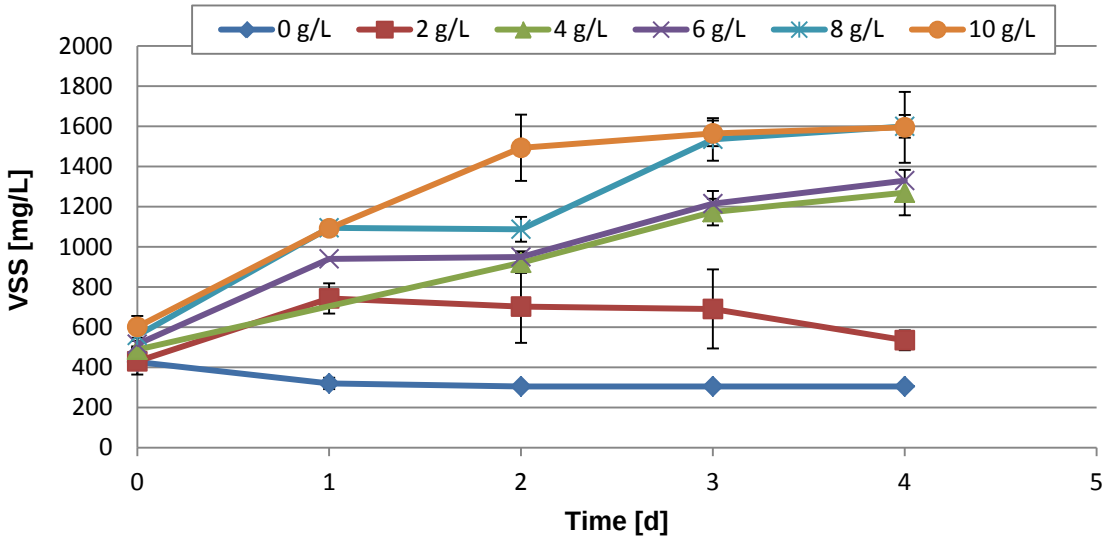

Figure 7.1. *Activated sludge, the 1st initial glucose addition experiment: VSS during cultivation.*

As seen in the Figure 7.2, in the end of the experiment there was COD_s left in the batch bottles with initial glucose concentrations from 6 g/L to 10 g/L. Initial glucose concentration and initial COD_{tot} were approximately similar, although with the lowest initial glucose concentrations the COD_{tot} was higher than glucose concentration because the ratio of organic compounds in wastewater and sludge to the added glucose is higher. The average initial COD_{tot} and COD_s without glucose addition were 580 ± 30 mg/L and 55 ± 10 mg/L, respectively. The decrease in COD_{tot} was caused by biodegradation of glucose and cell respiration, which releases CO_2 in the air.

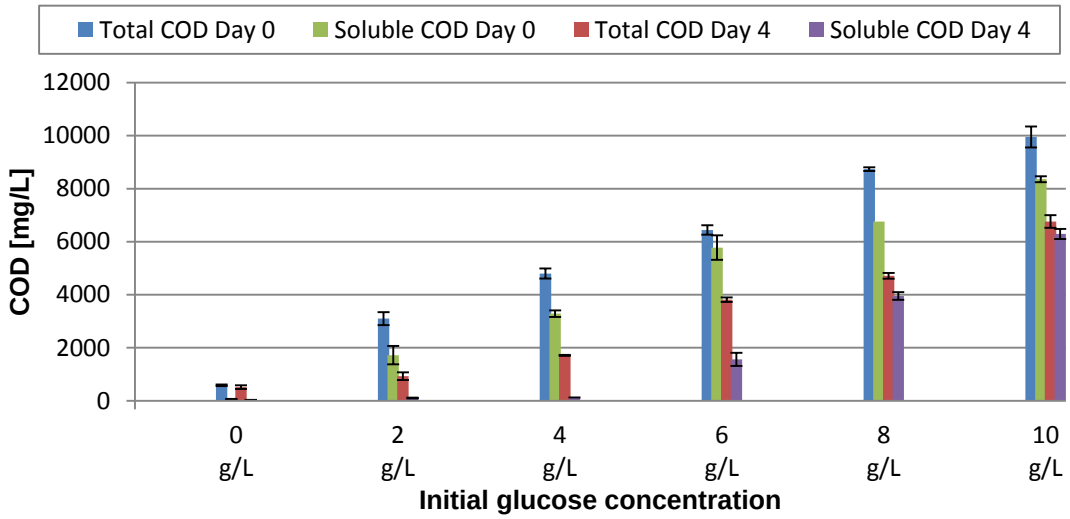

Figure 7.2. *Activated sludge, the 1st initial glucose addition experiment: COD at the start and in the end.*

The pH increased quite constantly from approximately 6.5 to 7.5 and even to 8.4 in the bottles with initial glucose concentration of 2 g/L. In some bottles, the DO decreased near 3 mg/L during the logarithmic growth phase.

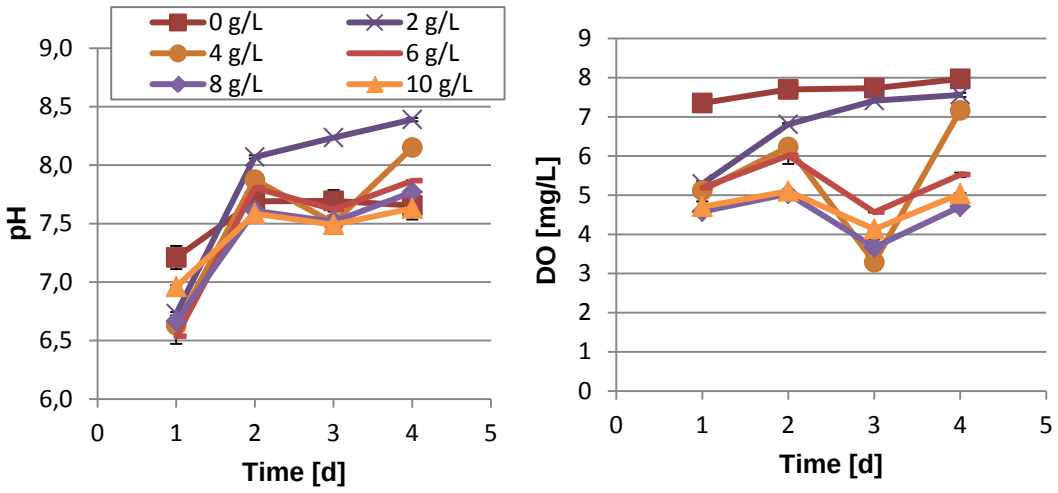

Figure 7.3. *Activated sludge, the 1st initial glucose addition experiment: pH and DO during cultivation.*

Table 7.1. *Activated sludge, the 1st initial glucose addition experiment: BOD_{7ATU} at the start (day 1) and in the end of the cultivation.*

Initial glucose concentration	BOD_{7ATU} [mg/L]	
	Day 1	End
0 g/L	300 ± 20	190 ± 30
2 g/L	950 ± 0	500 ± 30
4 g/L	2280 ± 70	700 ± 50
6 g/L	3560 ± 10*	2190 ± 20
8 g/L	3930 ± 0*	2860 ± 0*
10 g/L	3960 ± 10*	2870 ± 10*

* Dilution factor too low, may have weakened the result reliability

The BOD results were consistent with the COD results, as there was still biologically degradable material (glucose) left in the batch bottles with initial glucose concentrations from 6 g/L to 10 g/L. In the same bottles, the used dilution factor used in BOD analysis was too low, which may have weakened the result reliability.

7.1.2 Activated Sludge, the 2nd Initial Glucose Addition Experiment

In the 2nd initial glucose addition experiment, effects of different organic carbon loads on the biomass production and sludge composition were studied. The COD_s was consumed quite steadily, as presented in Figure 7.4. As seen in Figure 7.5, the average COD_s consumption rates at the logarithmic growth phase (last day of the cultivations eliminated) varied slightly between batch bottles being highest in the bottles with initial glucose concentration of 8 g/L.

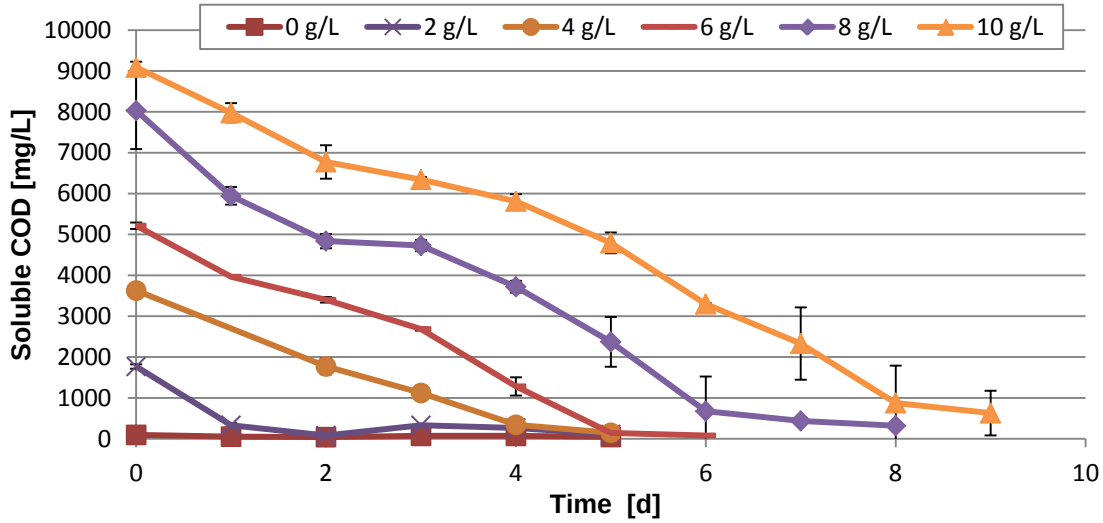

Figure 7.4. *Activated sludge, the 2nd initial glucose addition experiment: COD_s consumption.*

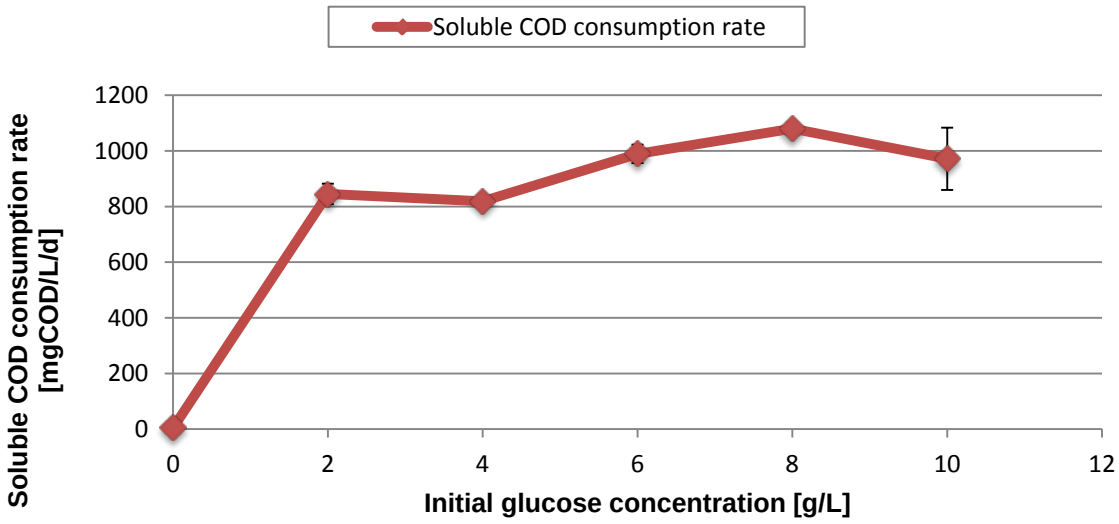

Figure 7.5. *Activated sludge, the 2nd initial glucose addition experiment: average COD_s consumption rates at the logarithmic growth phase.*

The VSS concentrations were as presented in Figure 7.6. The average initial VSS concentration was 550 ± 70 mg/L, approximately 50 mg/L higher than in the previous experiment. Without few exceptions, the biomass concentration increase was proportional to the initial glucose concentration in the bottles. In the other bottle with initial glucose concentration of 10 g/L, the VSS concentration in the end of the cultivation was significantly lower than the VSS in the parallel bottle. As seen in the Figure 7.8, the pH in the bottle with poor biomass increase was also different compared to the others. While pH in the other 11 bottles remained approximately between 7.0 and 8.0, the pH in the other 10 g/L bottle sunk even below 3.0, but rose near 8.0 in the end of the cultivation.

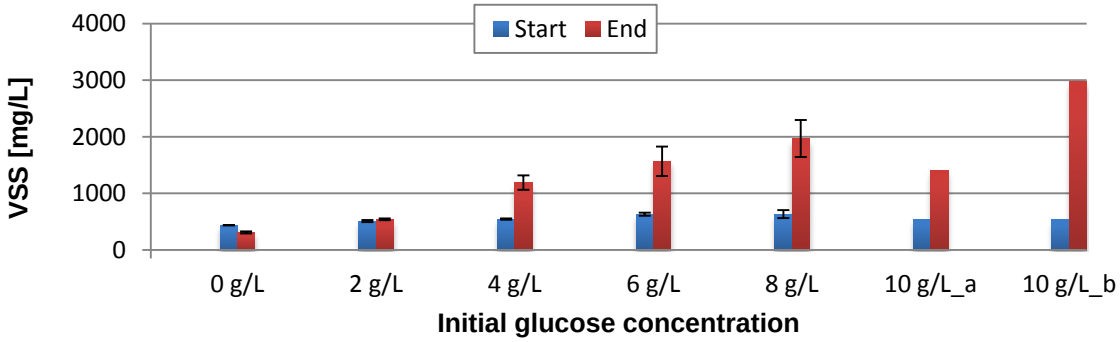

Figure 7.6. *Activated sludge, the 2nd initial glucose addition experiment: VSS at the start and in the end.*

The COD_{tot} was consumed by biodegradation of glucose and cell respiration, as seen in the Figure 7.7. Initial glucose concentration and initial COD_{tot} were approximately similar, although with the lowest initial glucose concentrations the COD_{tot} was higher than glucose concentration due to organic compounds already present in wastewater and sludge. The average initial COD_{tot} and COD_s without glucose addition were 640 ± 30 mg/L and 100 ± 30 mg/L, respectively.

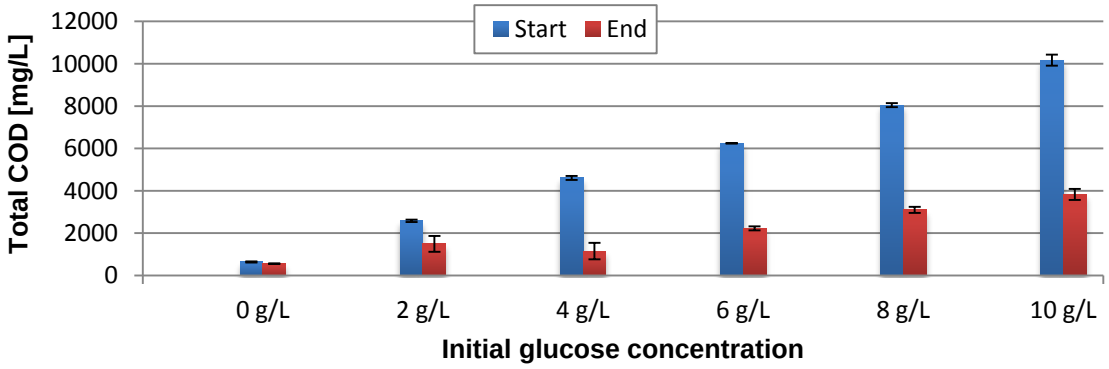

Figure 7.7. *Activated sludge, the 2nd initial glucose addition experiment: COD_{tot} at the start, in the end.*

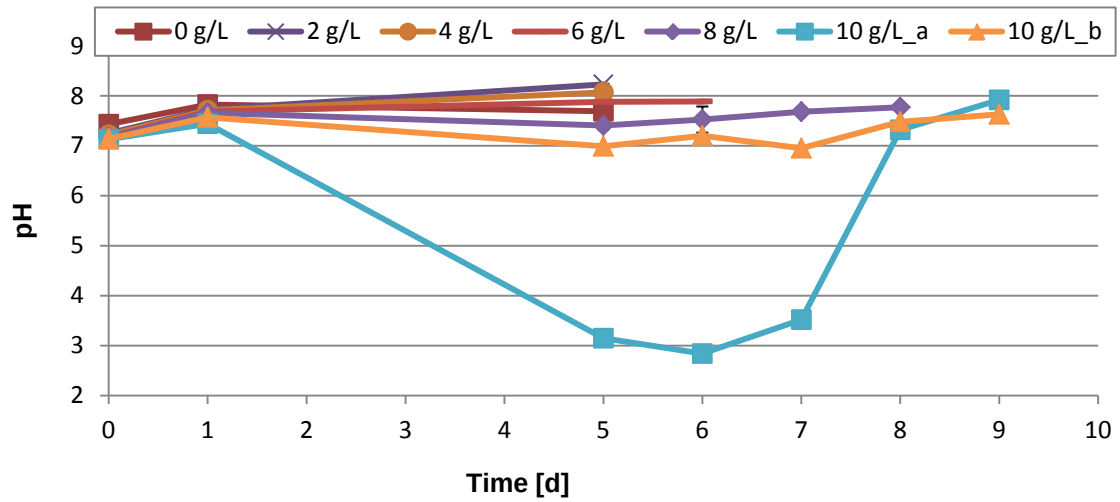

Figure 7.8. *Activated sludge, the 2nd initial glucose addition experiment: pH during the cultivation.*

The DO concentrations were as seen in Figure 7.9. During the logarithmic growth phase, DO decreased below 2 mg/L in the bottles with 10 g/L initial glucose concentration. Quite large deviations in measured DO concentrations were detected between parallel batch bottles.

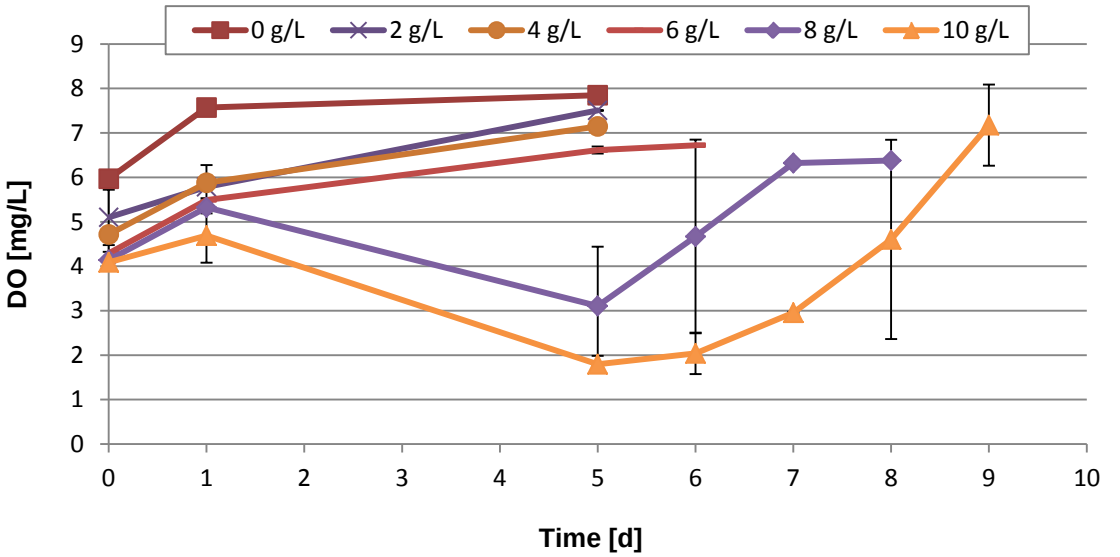

Figure 7.9. *Activated sludge, the 2nd initial glucose addition experiment: DO during the cultivation.*

The lipid content and concentration of extracted samples from selected bottles at the end of the cultivation were as presented in Figure 7.10.

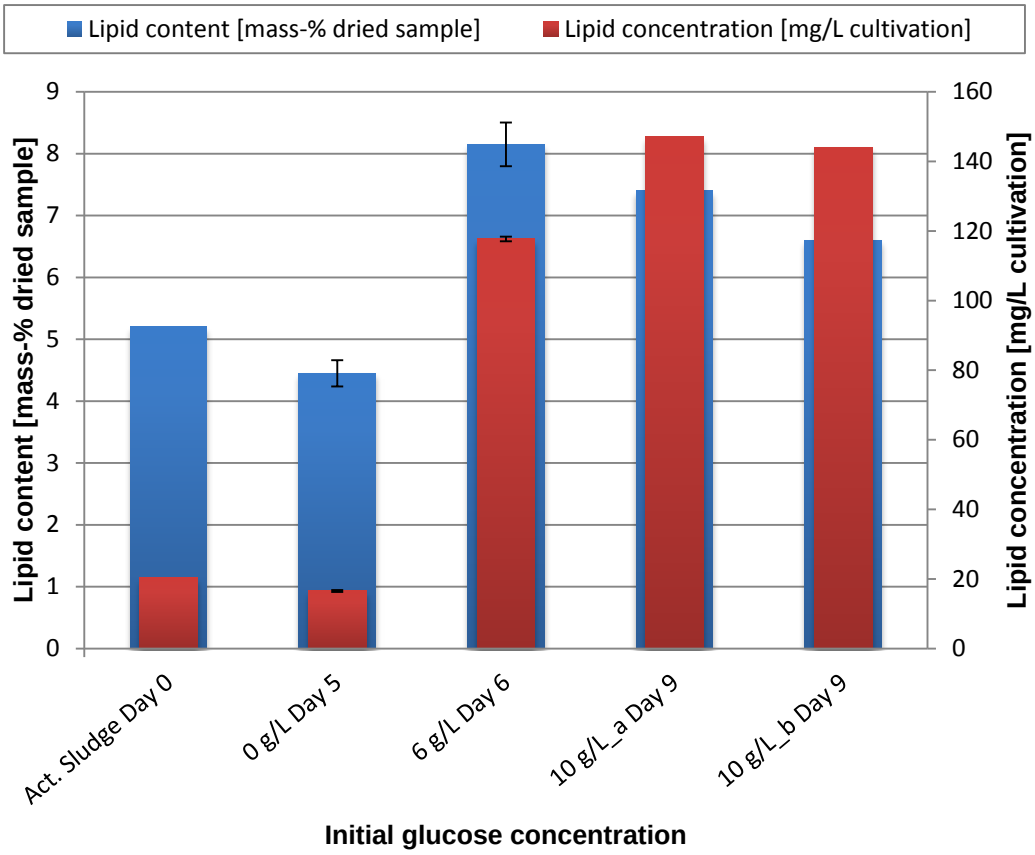

Figure 7.10. *Activated sludge, the 2nd initial glucose addition experiment: lipids.*

The activated sludge from Viinikanlahti WWTP contained approximately 5 mass-% and 20 mg/L lipids. When organic substrate was not available, the average lipid content after the cultivation was slightly lower than in the activated sludge (approx. 4.5 ± 0.2 mass-% and 17 ± 0 mg/L). Increase in lipid content and concentration to 8.2 ± 0.4 mass-% and 118 ± 1 mg/L was detected in the bottles with 6 g/L initially added glucose. Due to higher final biomass concentration, bottle a with 10 g/L initial glucose addition resulted he highest concentration of lipids, 147 mg/L.

7.2 Batch Bottle Experiments with Municipal Wastewater and Excess Sludge

Three batch bottle experiments were conducted with municipal wastewater and excess sludge. Effects of different organic carbon loads, different mixing rate, and low temperature on the biomass production and sludge composition were studied. In order to simulate higher organic carbon load, the C/N ratio was elevated by adding different concentrations of glucose into the batch bottles.

7.2.1 Excess Sludge, the 1st Initial Glucose Addition Experiment

In the 1st initial glucose addition experiment with municipal wastewater and excess sludge, the COD_s was consumed as presented in Figure 7.11. As seen in Figure 7.12, the average COD_s consumption rates at the logarithmic growth phase (stationary phase eliminated) varied slightly between batch bottles, but overall the COD_s consumption rates were approximately 400-600 mg/COD_s/L/d higher than with activated sludge used in the previous experiment.

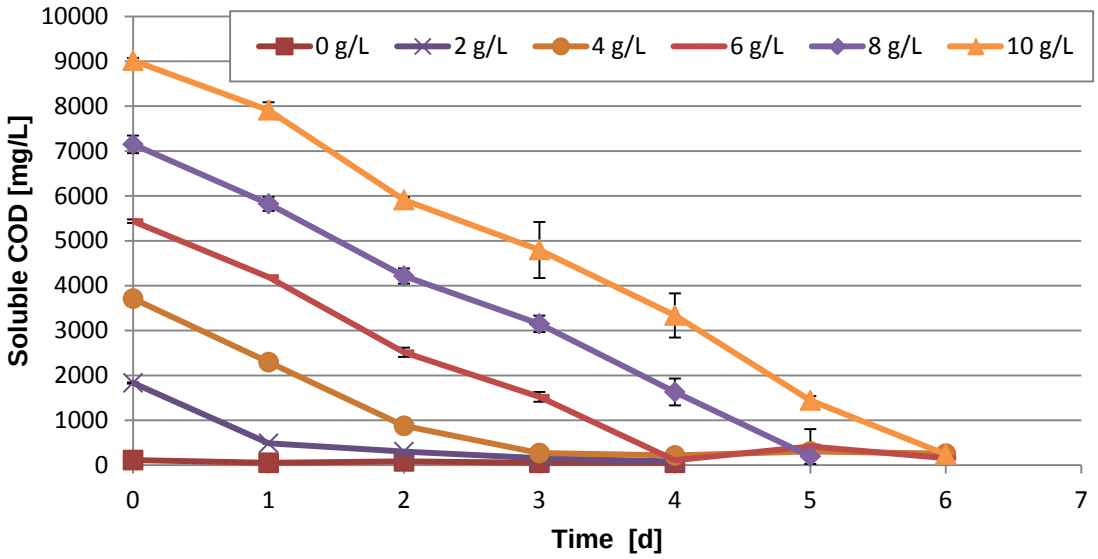

Figure 7.11. *Excess sludge, the 1st initial glucose addition experiment: COD_s consumption.*

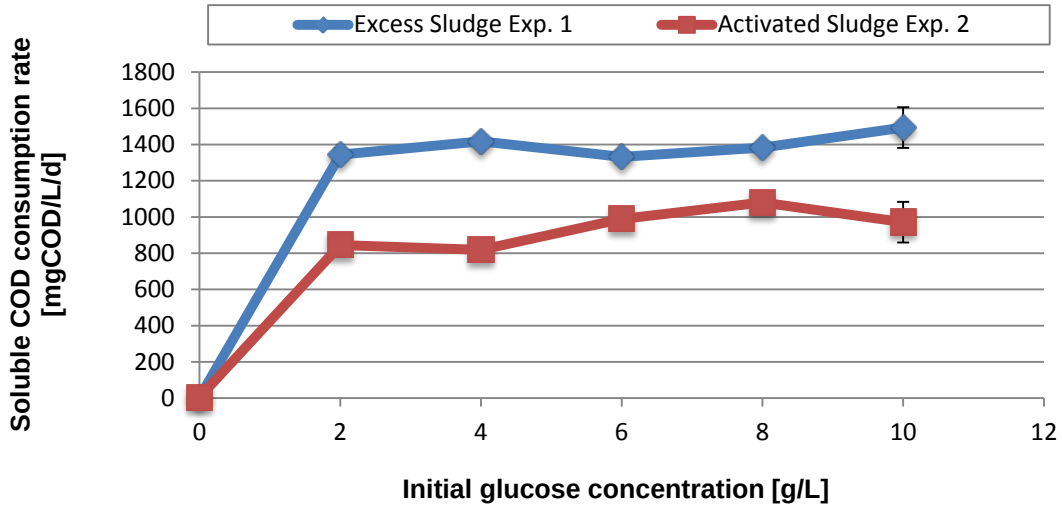

Figure 7.12. *The 1st initial glucose addition experiment for excess sludge and the 2nd initial glucose addition experiment for activated sludge; the average COD_s consumption rates at the logarithmic growth phases.*

The VSS concentrations were as presented in Figure 7.13. In the beginning of the experiment, the average VSS concentration was 940 ± 160 mg/L, approximately twice the VSS in the 2nd activated sludge experiment (Figure 7.6), but the resulting VSS concentrations did not differ as much, only 120 mg/L at the most. Cultivation after the initial glucose had run out may have decreased the VSS concentration from the maximum because biomass has been used for maintaining bacterial life.

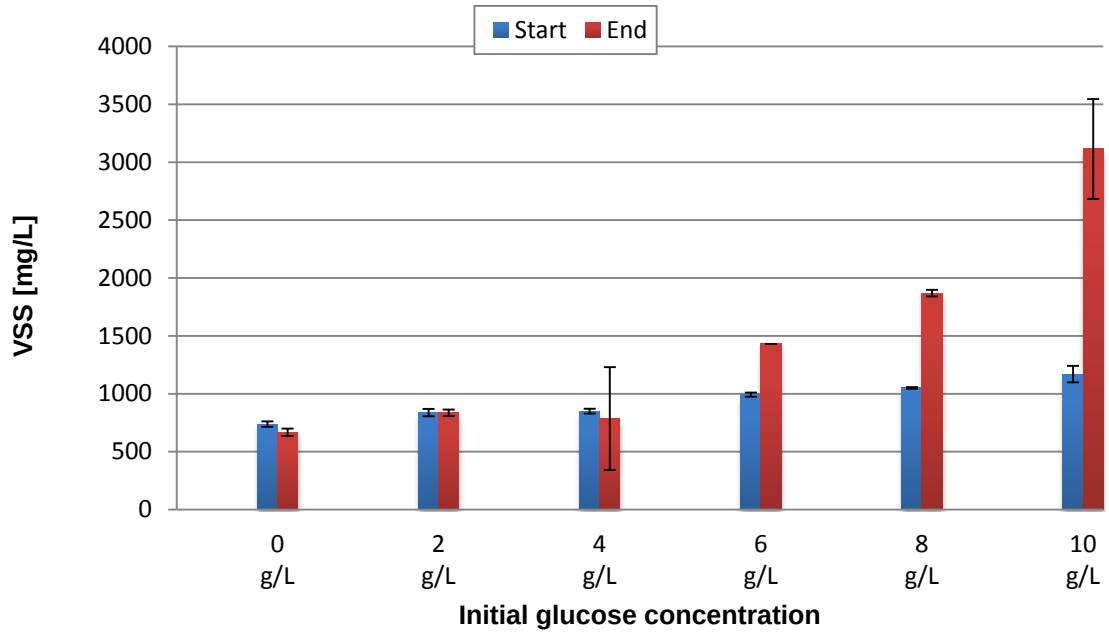

Figure 7.13. *Excess sludge, the 1st initial glucose addition experiment: VSS at the start and in the end.*

As in previous experiments, the COD_{tot} was consumed by biodegradation of glucose and cell respiration, as seen in Figure 7.14.

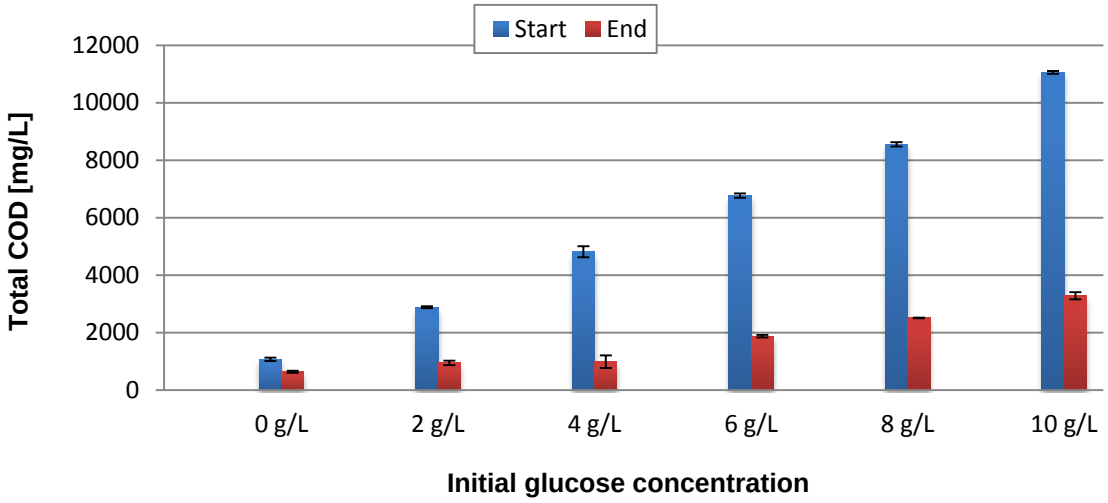

Figure 7.14. *Excess sludge, the 1st initial glucose addition experiment: COD_{tot} at the start, in the end.*

The pH in the cultivation bottles remained between 7.0 and 8.0, as presented in Figure 7.15.

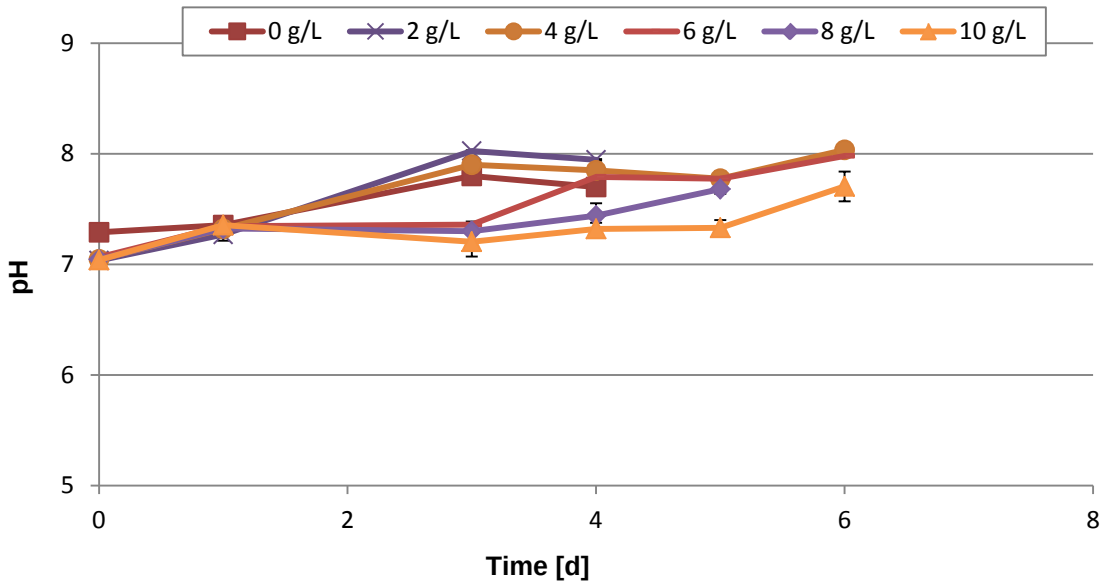

Figure 7.15. *Excess sludge, the 1st initial glucose addition experiment: pH during the cultivation.*

The dissolved oxygen concentrations were as seen in Figure 7.16. During the logarithmic growth phase, the DO sunk in every bottle with initially added glucose. The lowest DO concentration, 1.0 mg/L, was measured from the bottle with 8 g/L initially added glucose. Quite large deviations in measured DO concentrations were detected between parallel batch bottles.

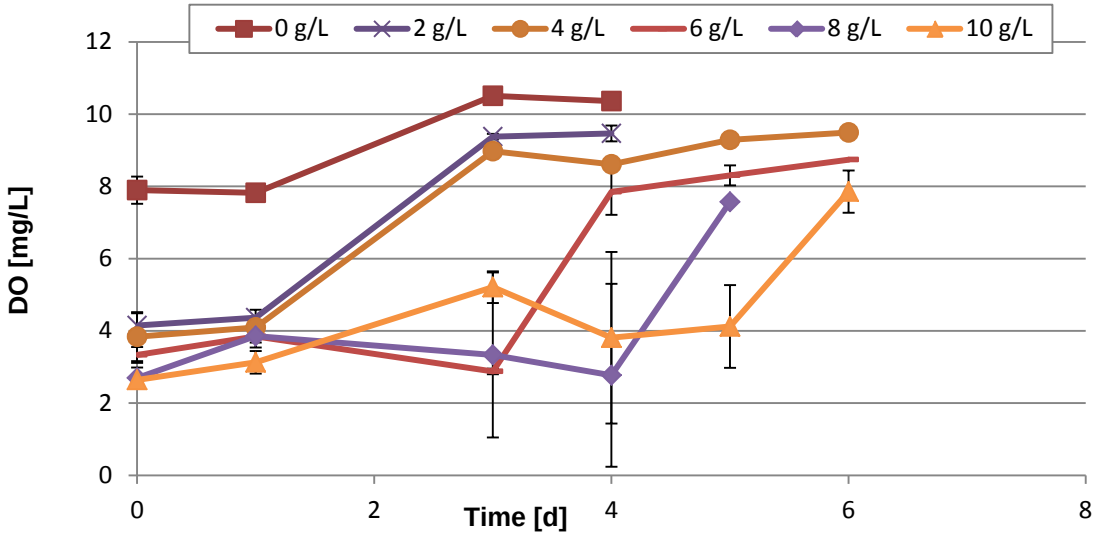

Figure 7.16. *Excess sludge, the 1st initial glucose addition experiment: DO during the cultivation.*

The lipid contents and concentrations of the extracted samples at the end of the cultivation were as presented in Figure 7.17.

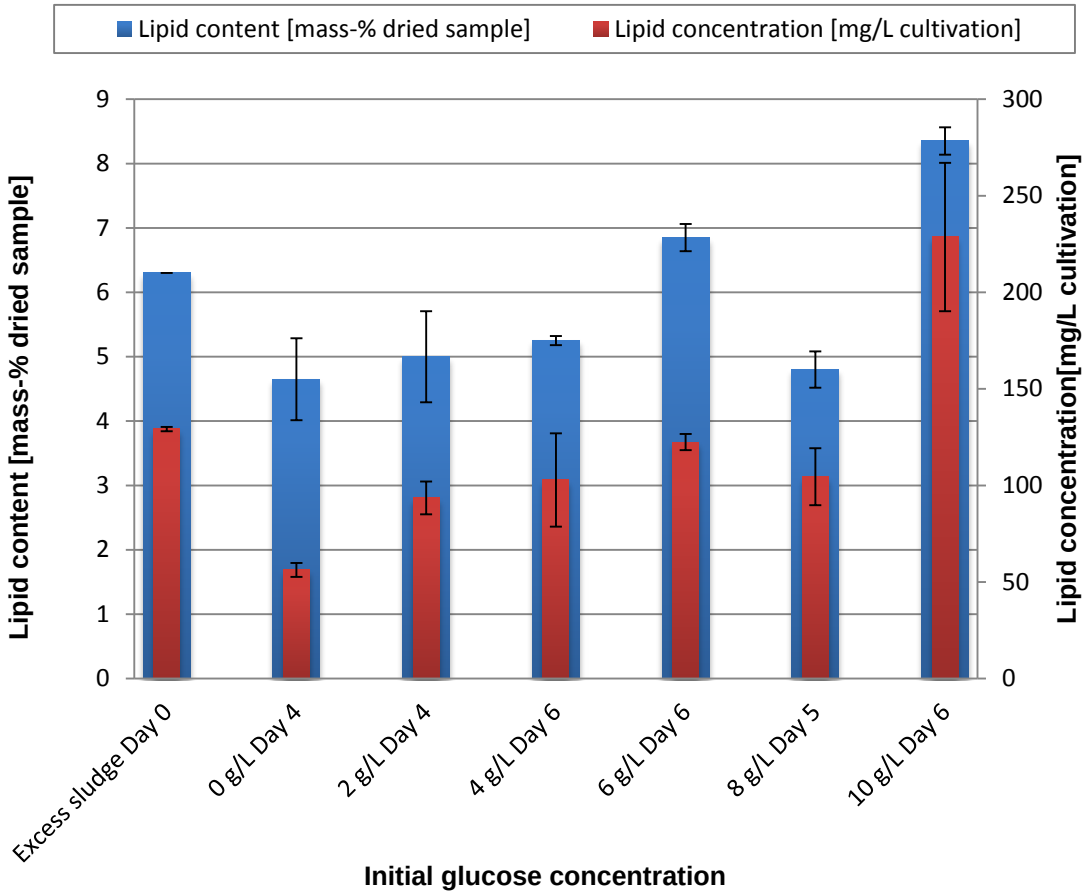

Figure 7.17. *Excess sludge, the 1st initial glucose addition experiment: lipids.*

The excess sludge from Viinikanlahti WWTP contained approximately 6.3 ± 0 mass-% and 129 ± 1 mg/L lipids. When organic substrate (glucose) was not added, the average lipid content and concentrations after the cultivation were lower than in the excess sludge (approx. 4.7 ± 0.6 mass-% and 56 ± 4 mg/L). Increase in lipid content and concentration to 8.4 ± 0 mass-% and 229 ± 38 mg/L was detected in the bottles with 10 g/L initially added glucose.

7.2.2 Excess Sludge, the 2nd Initial Glucose Addition Experiment, 300 rpm Mixing

In the 2nd initial glucose addition experiment, 300 rpm mixing rate was tested in the bottles with 8 g/L and 10 g/L initial glucose concentrations. Effects of different organic carbon loads and different mixing rate on the biomass production and sludge composition were studied.

The COD_s was consumed as presented in Figure 7.18. As seen in Figure 7.19, the average COD_s consumption rates at the logarithmic growth phase (stationary phase eliminated) varied slightly between batch bottles, but in the bottles with 300 rpm mixing rate, the COD_s consumption rates were 400-600 mg/COD_s/L/d lower than with 150 rpm mixing.

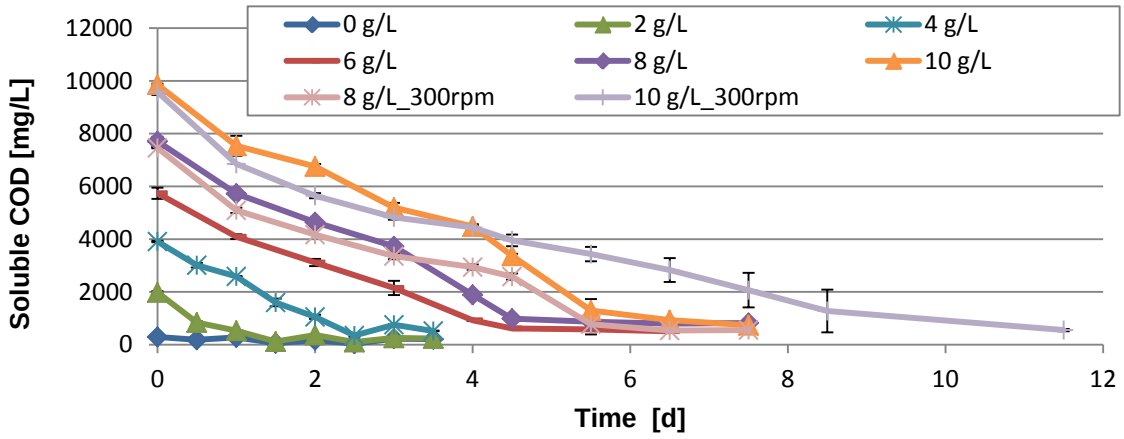

Figure 7.18. *Excess sludge, the 2nd initial glucose addition experiment: COD_s consumption.*

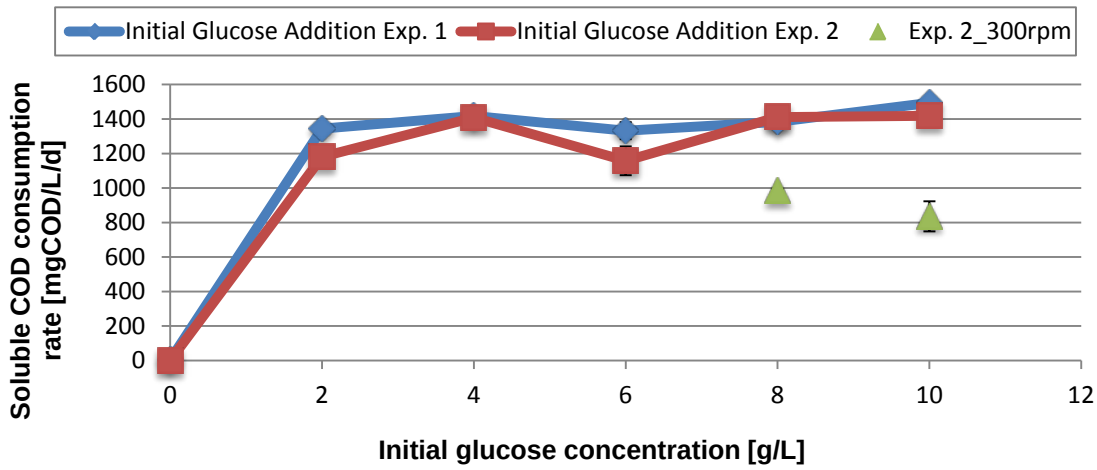

Figure 7.19. *The 1st and the 2nd initial glucose addition experiments for excess sludge; the average COD_s consumption rates at the logarithmic growth phases.*

The VSS concentrations were as presented in Figure 7.20. In the beginning of the experiment, the average VSS concentration was 2900 ± 230 mg/L, which is nearly three times the average initial VSS in the previous experiment (Figure 7.13). Also the VSS concentrations in the end of the experiment were different from the previous; increase in VSS was detected only in the bottles with higher mixing rate, and the increase was relatively small compared to previous experiments, only 200 ± 50 mg/L at the highest. In the other bottles, the VSS decreased approximately 100 – 1 000 mg/L.

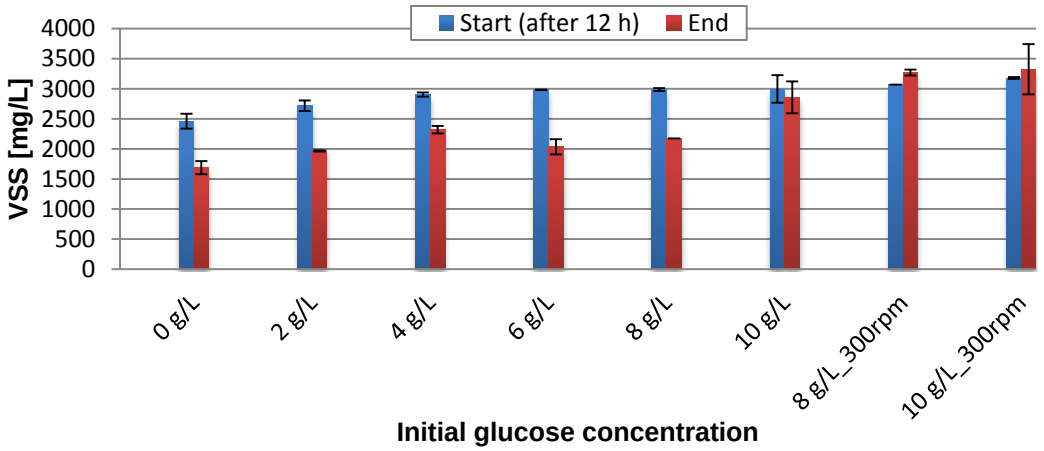

Figure 7.20. *Excess sludge, the 2nd initial glucose addition experiment: VSS at the start and in the end.*

The COD_{tot} was as presented in Figure 7.21. The initial COD_{tot} in the bottles with 10 g/L glucose addition was over 2 000 mg/L higher in the bottles with 150 rpm mixing rate than the ones with 300 rpm mixing, although sampling was done immediately after glucose addition and the COD_s were approximately similar.

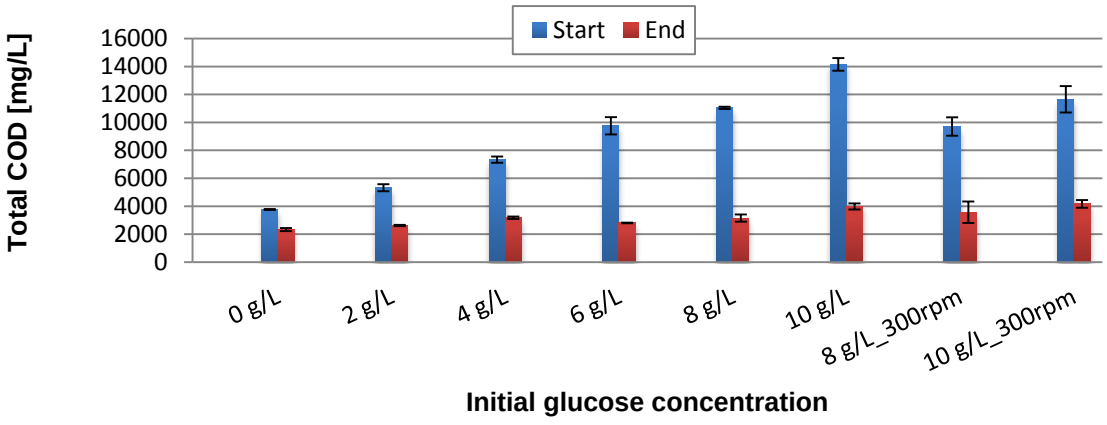

Figure 7.21. *Excess sludge, the 2nd initial glucose addition experiment: COD_{tot} at the start, in the end.*

The initial total nitrogen concentrations and initial C/N ratios in the 2nd activated sludge experiment and the 1st and the 2nd excess sludge experiments were as presented in Table 7.2. The C/N ratios are expressed as COD_s/N_{tot} in order to express the ratio of soluble organic substrate to total nitrogen. In the 2nd excess sludge experiment the total nitrogen concentration was over two times higher than in the two previous experiments. Naturally, the higher N_{tot} concentrations lead to lower C/N ratios when glucose was the main source of COD_s.

Table 7.2. *The 2nd initial glucose addition experiment for activated sludge, and the 1st and the 2nd initial glucose addition experiments for excess sludge, C/N ratios as COD_s/N_{tot} and total nitrogen concentrations in the beginning of the cultivations.*

Initial glucose [g/L]	N_{tot} [mg/L]*			C/N ratio [COD_s/N_{tot}]		
	Act. Sludge Exp. 2	Exc. Sludge Exp. 1	Exc. Sludge Exp. 2	Act. Sludge Exp. 2	Exc. Sludge Exp. 1	Exc. Sludge Exp. 2
10	59	60	130	155 : 1	151 : 1	76 : 1
8				137 : 1	120 : 1	59 : 1
6				89 : 1	91 : 1	44 : 1
4				62 : 1	62 : 1	30 : 1
2				30 : 1	31 : 1	15 : 1
0				2 : 1	2 : 1	2 : 1

* As nothing else than glucose was added into the cultivation bottles, the N_{tot} concentration is approximated to be similar in every bottle.

In every bottle from 4 g/L initial glucose addition, the pH dropped as presented in Figure 7.22. The lowest detected pH was 2.9 in the bottle with 10 g/L initial glucose addition and 300 rpm mixing rate. The pH rose towards the end of the cultivation (towards the end of the logarithmic growth). The initial pH was not measured, but it can be estimated to have been between 7.0 and 8.0 based on the previous experiments and the pH in the bottles with no added glucose.

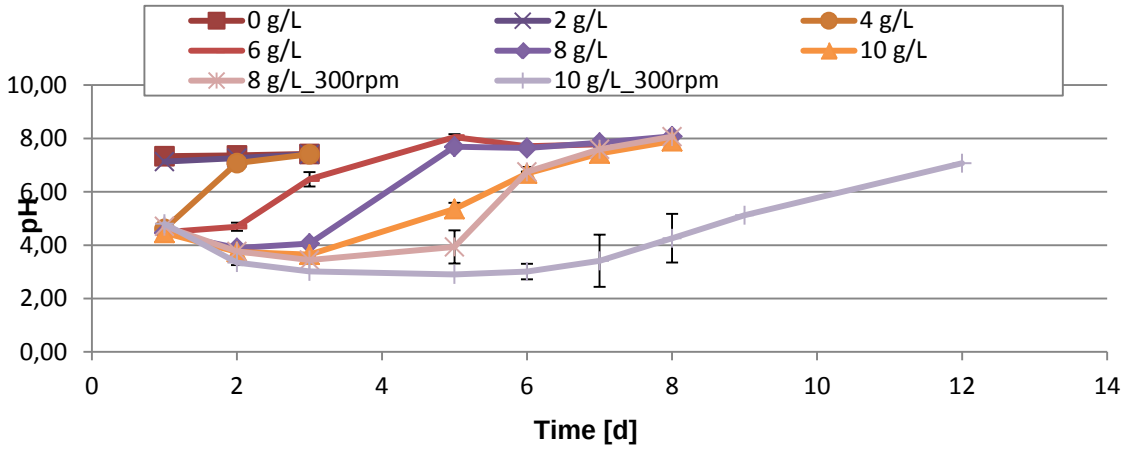

Figure 7.22. *Excess sludge, the 2nd initial glucose addition experiment: pH during the cultivation.*

In every bottle where initial glucose was added and the mixing rate was 150 rpm, the DO went near 0 mg/L during the logarithmic growth phase, as presented in Figure 7.23. The 300 rpm mixing was enough to maintain the DO above 4.5 mg/L.

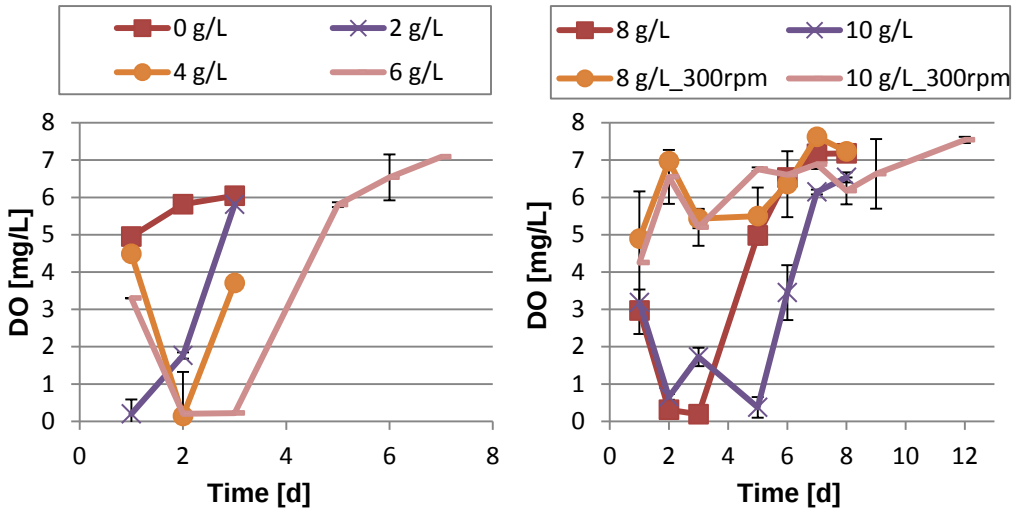

Figure 7.23. *Excess sludge, the 2nd initial glucose addition experiment: DO during the cultivation.*

Figure 7.24 shows that the lipid content and concentration of the extracted samples varied largely even between parallel cultivation bottles.

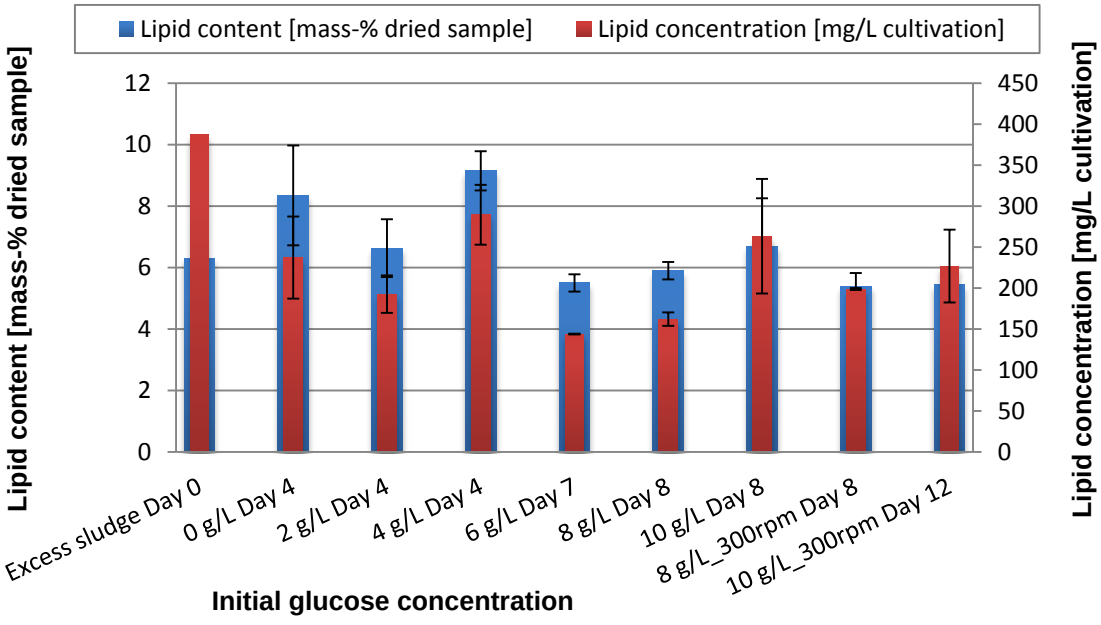

Figure 7.24. *Excess sludge, the 2nd initial glucose addition experiment: lipids.*

The same initial excess sludge lipid results than in the previous experiment were used (approx. 6.0 ± 0 mass-% in dried sample), but since the VSS was three times higher, lipid concentration was estimated to be 390 mg/L. Only minor decreases (0.2 – 1%) compared to the excess sludge lipids were detected in mass-% lipid content in biomass, but volumetric losses in lipid concentrations were larger, 240 mg/L at the highest, in the bottles with 6 g/L initial glucose concentration. The reason for decrease in lipid content and concentration may have been the prolonged cultivation. In absence of organic substrate (glucose), intracellular lipids have been used for maintaining the microbial life.

7.2.3 Excess Sludge, Initial Glucose Addition at Low Temperature

The third excess sludge experiment was conducted at 5.6 °C as compared to 27 °C in the other experiments. Based on the previous experiments, the orbital shaker was set at 300 rpm in order to get efficient aeration. The soluble COD was consumed as presented in Figure 7.25. The other bottle with 10 g/L initial glucose concentration fell of the orbital shaker and broke on the 4th day of the experiment. As seen in Figure 7.26, the average COD_s consumption rates at the logarithmic growth phase (stationary phase eliminated) were approximately 950 mg/COD_s/L/d lower at 5.6 °C than at 27 °C.

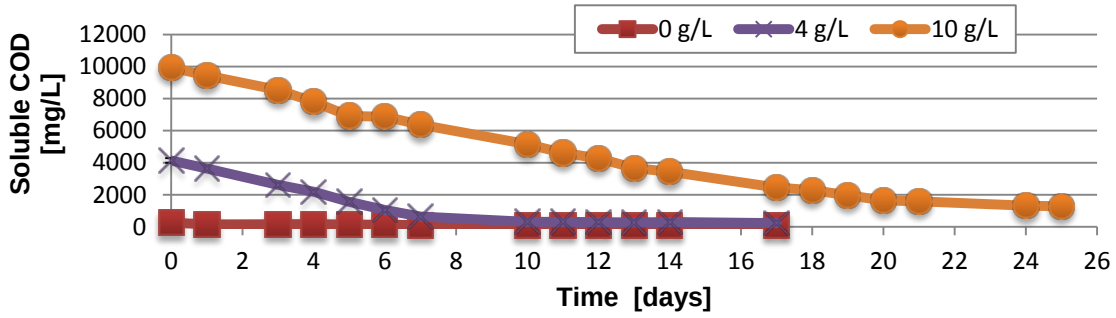

Figure 7.25. *Excess sludge, initial glucose addition at low temperature: COD_s consumption.*

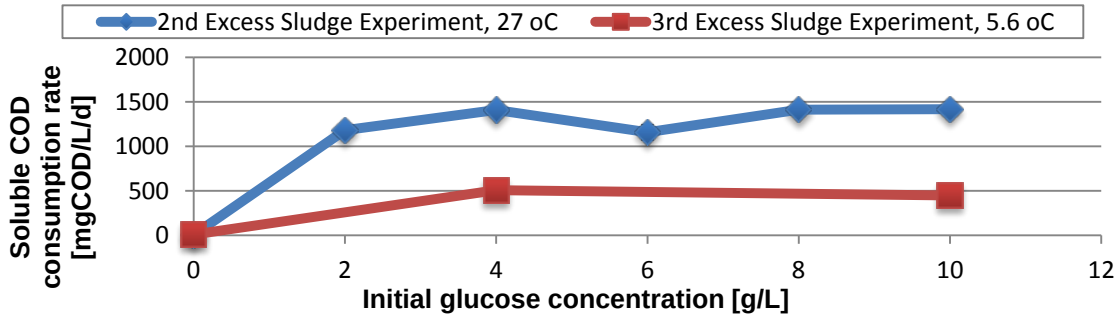

Figure 7.26. *The 2nd and the 3rd initial glucose addition experiments for excess sludge, at 27 °C and 5.6 °C, respectively. The average COD_s consumption rates at the logarithmic growth phases.*

The VSS concentrations were as presented in Figure 7.27. The average initial VSS concentration was 3 600 ± 650 mg/L, which was 700 mg/L higher than in the previous experiment. In the bottles with 4 g/L initial glucose concentration, the VSS concentration increased only slightly (300 mg/L) in the other bottle. Six days after the added glucose (COD_s) had been consumed the VSS was approximately 400 mg/L lower than the initial. In the 10 g/L bottle VSS increased approximately 1 300 mg/L during glucose consumption. Even four days after the added glucose had been consumed the VSS had increased 100 mg/L.

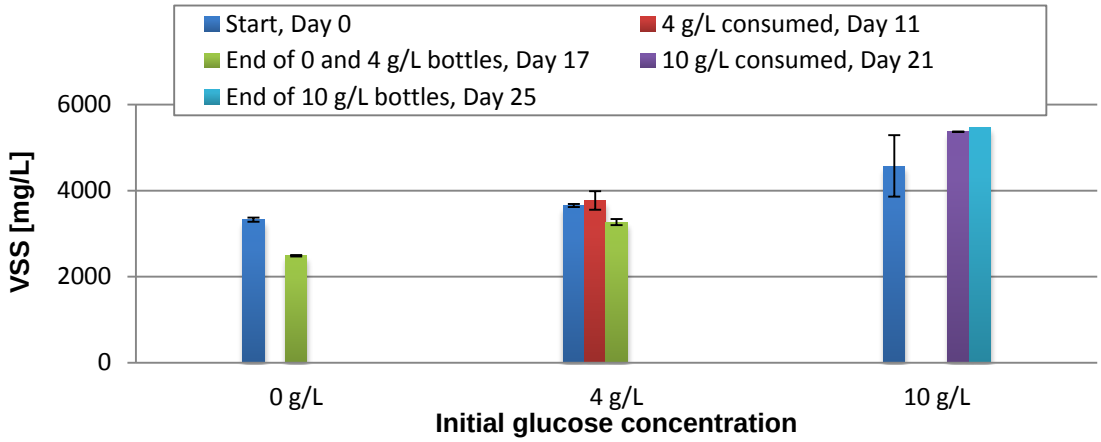

Figure 7.27. *Excess sludge, initial glucose addition at low temperature: VSS concentrations at the start, after the added glucose (COD_s) had been consumed and in the end of the cultivation.*

The pH remained between 7.0 and 8.5 as presented in Figure 7.28. At the lowest, pH was 7.18 in the bottle with 10 g/L initial glucose addition. When glucose had been consumed, pH slowly began to rise in every bottle.

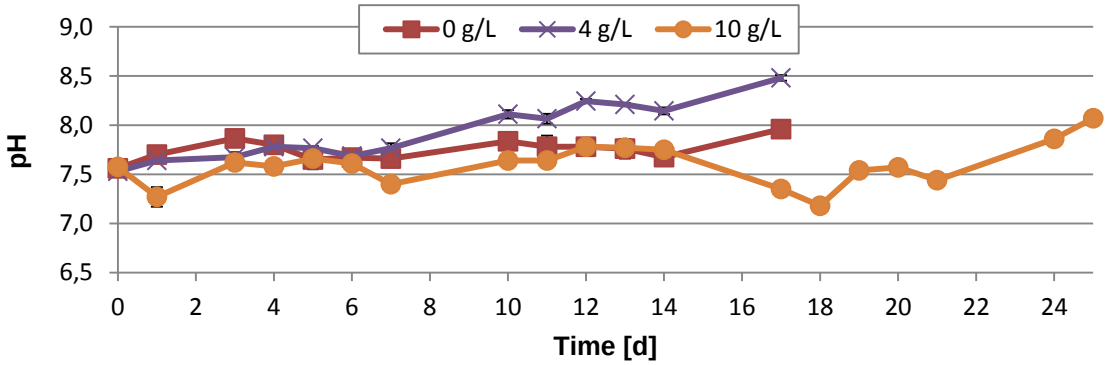

Figure 7.28. *Excess sludge, initial glucose addition at low temperature: pH during the cultivation*

Lower cultivation temperature kept the COD_s consumption rate low enough to keep the DO above 8 mg/L in every bottle as presented in Figure 7.29.

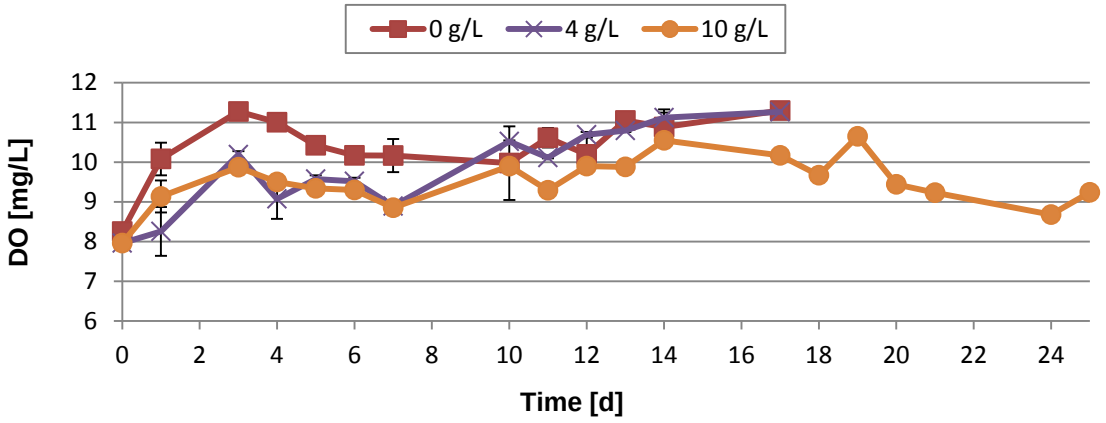

Figure 7.29. *Excess sludge, initial glucose addition at low temperature: DO during the cultivation*

The lipid contents and concentrations of the samples extracted during and at the end of the cultivation were as presented in Figure 7.30.

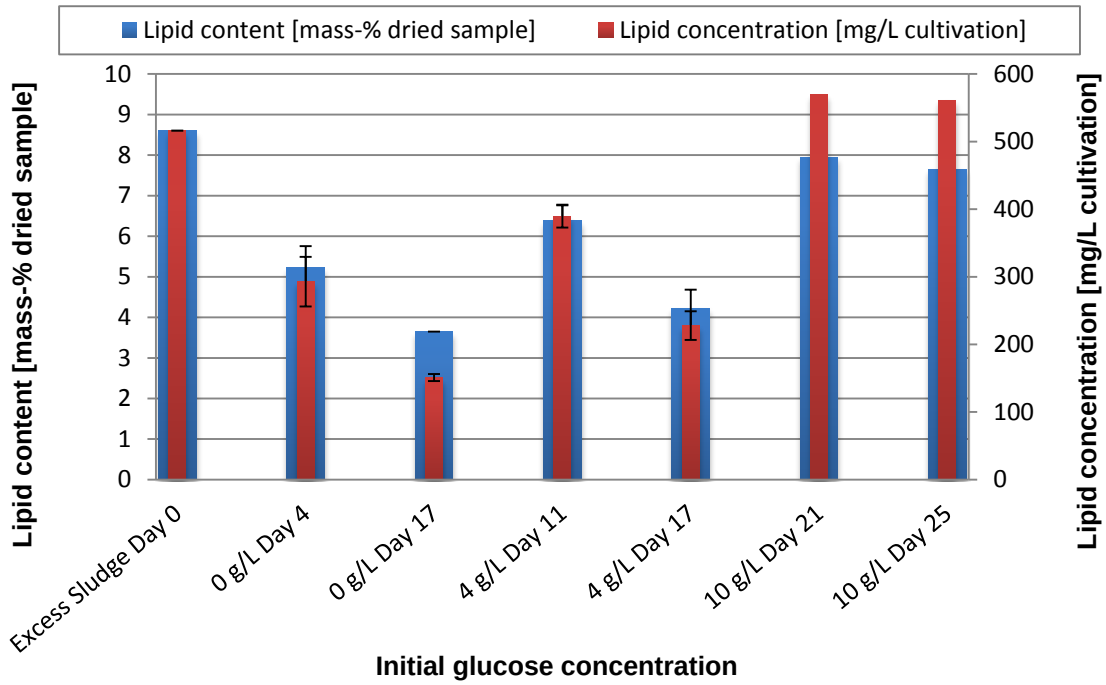

Figure 7.30. *Excess sludge, initial glucose addition at low temperature: lipids.*

The excess sludge from Viinikanlahti WWTP contained approximately 8.6 mass-% (dried sample) and 520 mg/L (cultivation) lipids, which was higher than the initial excess sludge lipid results in previous experiments (6.0 mass-%.). The lipid content decreased from initial in almost every sampling point, only volumetric content slightly increased in the 10 g/L bottles. The greatest decrease was achieved on day 17 in the bottles with no added glucose, where the average lipid content and concentration were 3.7 ± 0.5 mass-% and 151 ± 5 mg/L.

7.3 Laboratory Scale Activated Sludge Reactor

7.3.1 1st Reactor Run

The 1st run with laboratory scale activated sludge reactor continued for 20 days. The MLSS concentration in the aeration tank was as presented in Figure 7.31. The COD_s of influent wastewater and reactor effluent, and COD_s reduction percentages were as presented in Figure 7.32. Error bars in the figures represent standard deviations of the replicate analysis results.

In the beginning of the run, MLSS concentration of the activated sludge from Viinikanlahti WWTP was approximately 6 100 mg/L. During the run, the MLSS declined near 5 100 mg/L, then rose near 5 700 mg/L and dropped again to 5 100 mg/L at the end of the run.

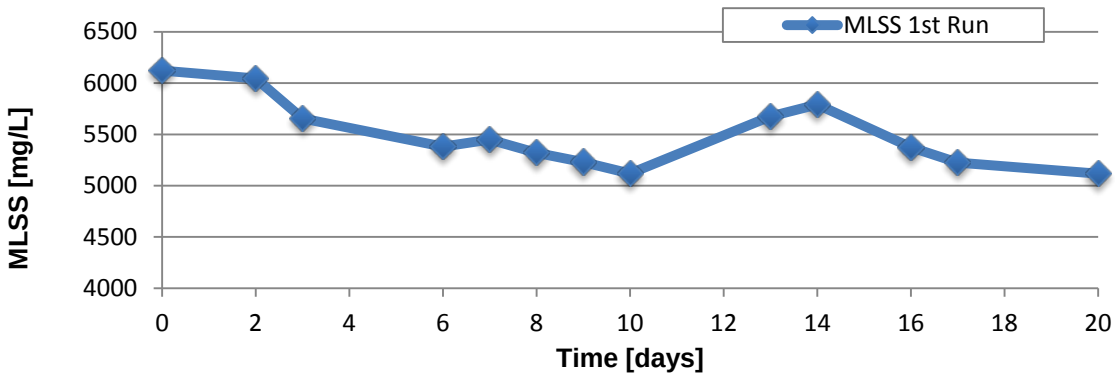

Figure 7.31. *Laboratory scale activated sludge reactor, 1st run MLSS.*

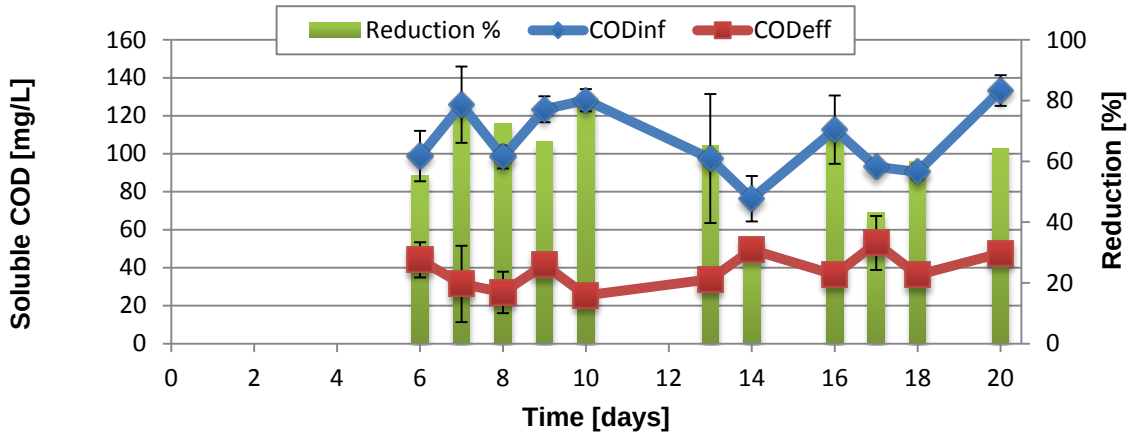

Figure 7.32. *Laboratory scale activated sludge reactor, 1st run COD_s reduction.*

During the 1st reactor run the average COD_s of influent and effluent were 107 mg/L and 39 mg/L, respectively. Some degradation occurred already in the influent canister during storing and pumping. Because of that, the influent COD_s varied quite heavily from 76 mg/L to 133 mg/L. The effluent COD_s varied from 31 mg/L to 53 mg/L. The average COD_s reduction percentage was 62%, being 80% at the highest and 35% at the lowest.

The COD_{tot} of influent wastewater and reactor effluent, and COD_{tot} reduction percentages were as presented in Figure 7.33. Biological degradation and settling of the solid particles during storing and pumping of the influent wastewater affected significantly the influent COD_{tot} and the organic load of the reactor. Between days 9 and 18, the influent COD_{tot} decreased from 350 mg/L to 120 mg/L, even though fresh wastewater was brought from Viinikanlahti twice a week. After 14 days the process began to fail, and solid organic particles were washed out from the reactor. In the end of the run, only 2.5% COD_{tot} reduction was obtained.

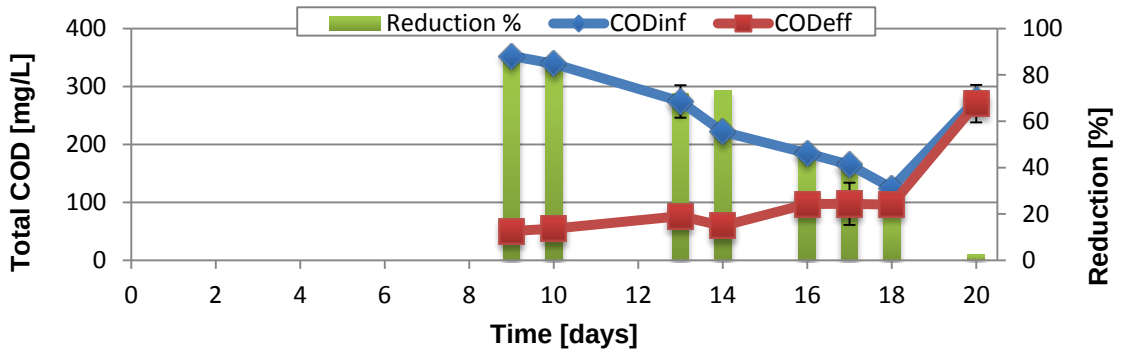

Figure 7.33. *Laboratory scale activated sludge reactor, 1st run COD_{tot} reduction.*

The pH and DO of activated sludge and influent wastewater were as presented in Figure 7.34. Different mixing and aeration solutions were experimented for the influent canister, which accelerated the degradation in the canister even more, but did not seem to

prevent the settling of the solid wastewater particles. The pH of activated sludge decreased during the whole reactor run from 6.8 to 5.0. Aeration was enough to maintain the DO above 5.5 mg/L and provide sufficient mixing.

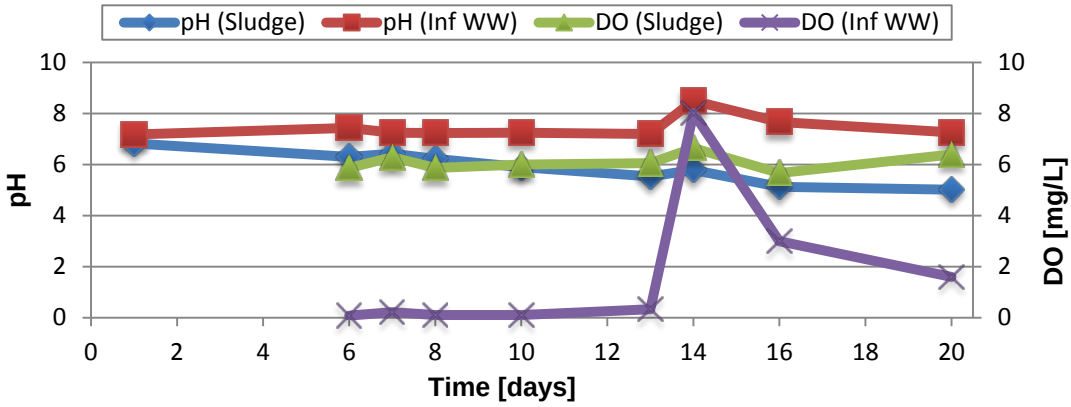

Figure 7.34. *Laboratory scale activated sludge reactor, 1st run pH and DO.*

The BOD_{7ATU} analysis according to Finnish Standard SFS 3019 (1979) was conducted on the 9th day of the reactor run. The BOD_{7ATU} for influent wastewater and reactor effluent were 94 ± 11 mg/L and 4 ± 1 mg/L, respectively. BOD_{7ATU} reduction was 96%. On the 9th day, the corresponding COD_s results were 123 ± 7 mg/L, 42 ± 2 mg/L and 66%, respectively.

7.3.2 2nd Reactor Run

The 2nd run with laboratory scale activated sludge reactor continued for 87 days. The MLSS concentration in the aeration tank was as presented in Figure 7.35. The total COD_s and COD_{tot} of influent wastewater and reactor effluent, and COD reduction percentages were as presented in Figure 7.36 and Figure 7.37, respectively.

In the beginning of the run, MLSS concentration of the activated sludge from Viinikanlahti WWTP was approximately 5000 mg/L, which is about 1 100 mg/L lower than in the 1st run. During the run, the MLSS declined quite steadily, being 1 600 mg/L at the lowest. Towards the end of the reactor run, the MLSS slightly increased because of the higher organic load, finishing at approximately 2 100 mg/L.

During the 2nd reactor run, the activated sludge process was quite stable. With conventional organic load before glucose addition, the effluent COD_s and COD_{tot} remained under 50 mg/L and 70 mg/L, respectively. The average effluent COD_s was 41 ± 10 mg/L and average effluent COD_{tot} was 56 ± 13 mg/L. The average reduction-% was 66 ± 7% for COD_s and 74 ± 6% for COD_{tot}. For comparison, the COD_s and COD_{tot} of Viinikanlahti effluent were analyzed once; both resulting 37 mg/L while COD_s and COD_{tot} reduction-% were 68% and 83%, respectively. After the reactor was introduced to the higher organic load, effluent COD started to rise, but COD reduction rates remained quite similar due to higher influent COD.

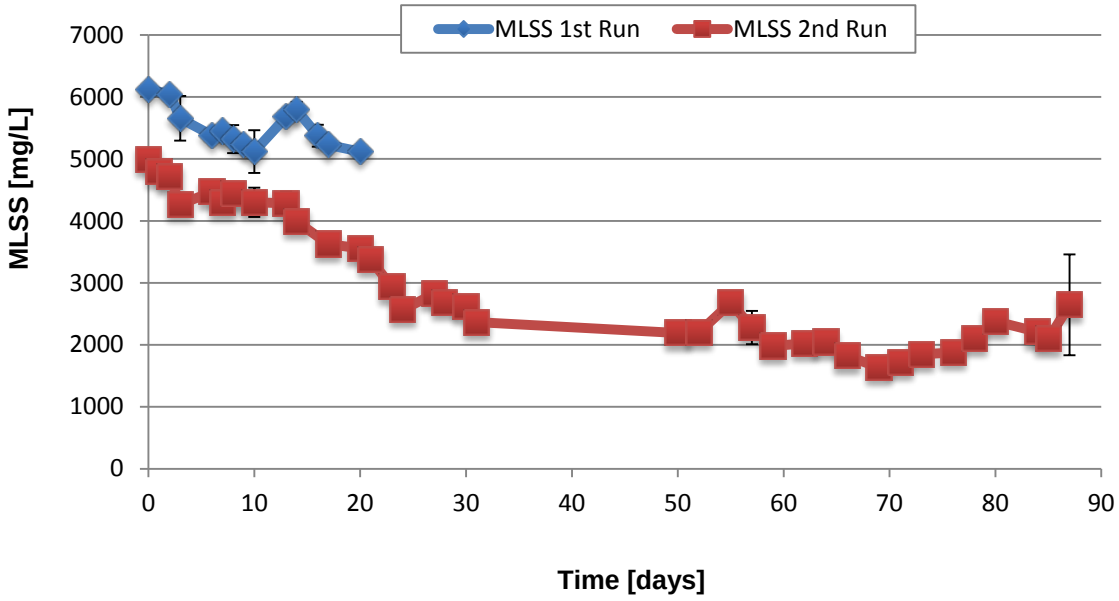

Figure 7.35. *Laboratory scale activated sludge reactor, 1st and 2nd run MLSS.*

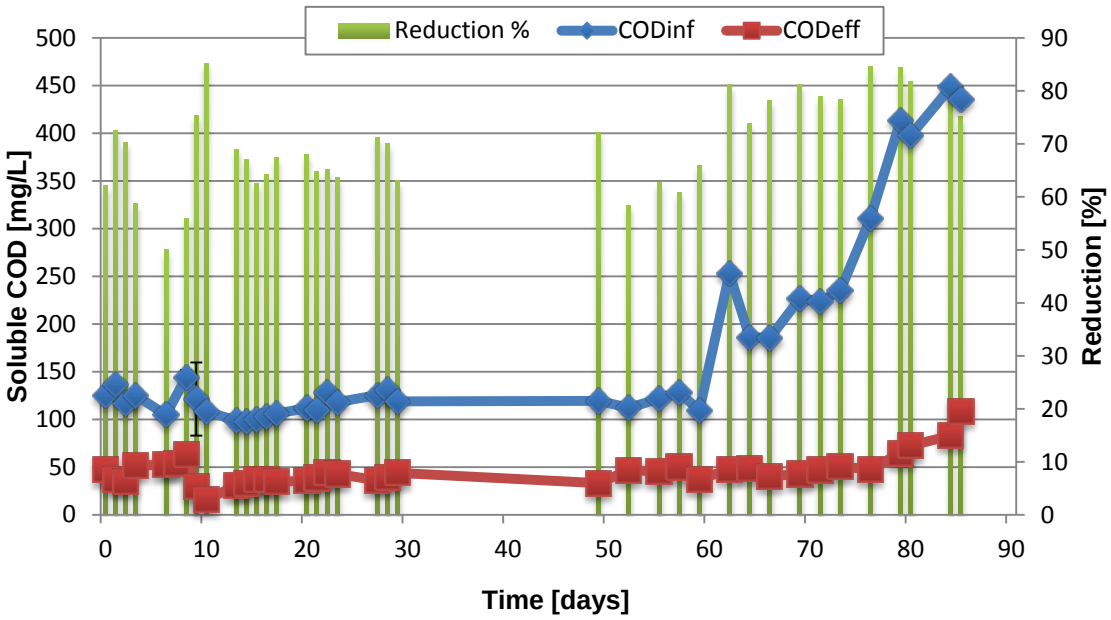

Figure 7.36. *Laboratory scale activated sludge reactor, 2nd run COD_s reduction.*

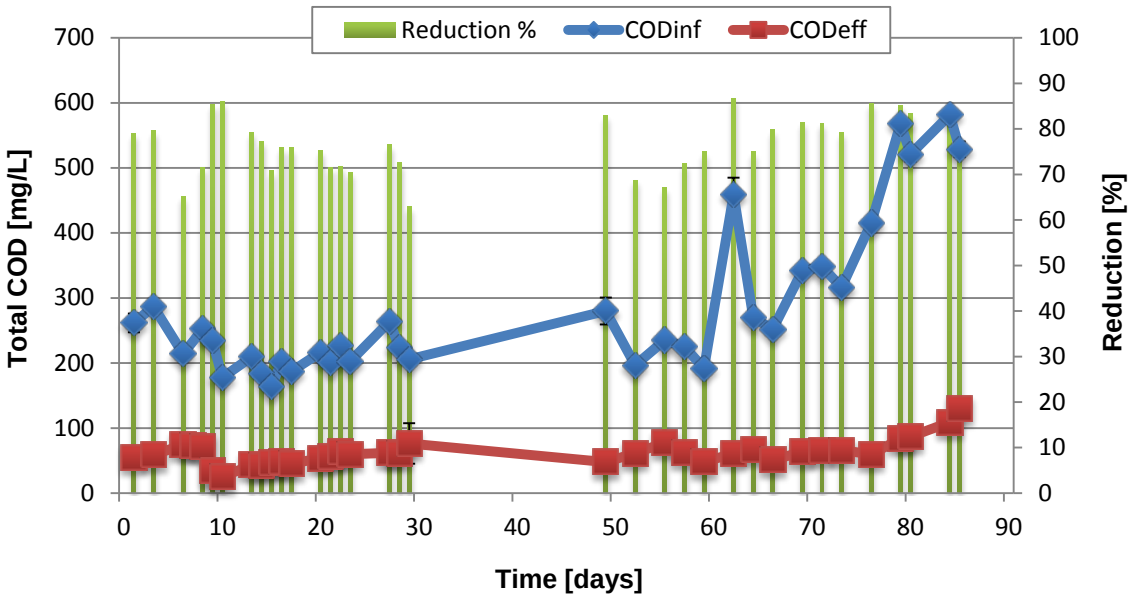

Figure 7.37. *Laboratory scale activated sludge reactor, 2nd run COD_{tot} reduction.*

The pH and DO of activated sludge and influent wastewater were as presented in Figure 7.38. Adding alkalinity (CaO) into the influent prevented the activated sludge pH from decreasing. The sludge pH was 5.8 at the lowest, and 7.5 at the highest. Aeration was enough to maintain the DO mainly above 4.5 mg/L and provide sufficient mixing. Influent pH and DO remained steadily near 7.3 and 0.5, respectively, and they were no longer measured after day 30.

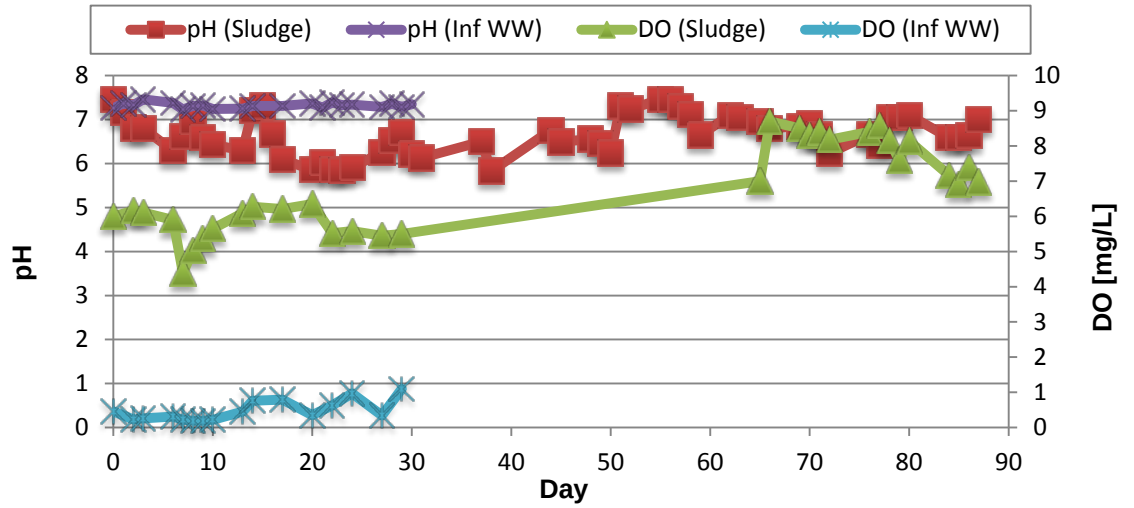

Figure 7.38. *Laboratory scale activated sludge reactor, 2nd run pH and DO.*

The BOD_{7ATU} analysis according to Finnish Standard SFS 3019 (1979) was conducted on the 22nd day of the reactor run. On the 24th day, BOD_{7ATU} was analyzed using WTWs OxiTop® Control BOD Respirometer System. For comparison, also effluent wastewater from Viinikanlahti WWTP was analyzed. Results were as presented in Table 7.3. On day 22, reactors BOD_{7ATU} reduction was 4.6 percentage units lower than in Viinikanlahti. On day 24 the corresponding number was 3.9.

Table 7.3. *Laboratory scale activated sludge reactor, 2nd run, and Viinikanlahti WWTP, BOD_{7ATU}.*

Day/Method	Sample	Average BOD_{7ATU} [mg/L]	BOD reduction [%]
22/SFS 3019	**Influent WW**	104 ± 1	
	Reactor effluent	7 ± 0	94
	Viinikanlahti effluent	2 ± 0	98
24/OxiTop®	**Influent WW**	138 ± 2	
	Reactor effluent	7 ± 5	95
	Viinikanlahti effluent	2 ± 0	99

On the 29th day of the reactor run, ammonium nitrogen was analyzed from the influent wastewater and reactor effluent. The average NH_4-N concentrations were 49 ± 1 mg/L and 6 ± 0 mg/L, respectively. Nitrification percentage was 88%. According to Sandelin (2010), the corresponding NH_4-N concentrations in Viinikanlahti WWTP (annual average) are approximately 35.0 mg/L and 0.04 mg/L (99.9% nitrification).

The organic load was increased in three steps: the COD_s concentration was set up 2, 3, and finally 4 times higher than the initial COD_s concentration of the influent wastewater. Before each step, lipid samples were taken from the activated sludge. Lipid contents and concentrations from extractions were as presented in Table 7.4.

Table 7.4. *Laboratory scale activated sludge reactor, 2nd run sludge lipids.*

Day	Influent ww C/N ratio [COD_s/N_{tot}]	Lipid content [mass-% dried sludge]	Lipid concentration [mg/L sludge]
0 (Act. Sludge)	-	5	200
59	2:1	7.1	102
73	5:1	6.5	132
87	9:1	7.0	150

Activated sludge from Viinikanlahti WWTP was used as initial sample for comparison. Higher organic loadings produced slightly more lipids in mass-%, but the mg/L sludge lipid concentration was lower than initial because of the lower MLSS of the laboratory scale reactor.

8 DISCUSSION

8.1 Batch Bottle Experiments with Municipal Wastewater, Activated or Excess Sludge

8.1.1 Substrate Utilization and Biomass Production

The average COD_s consumption rates at the logarithmic growth phases of each experiment were as presented in Figure 8.1. No shock effect or significant lag phase was detected in any of the experiments. On the contrary, despite the concentration of added glucose, the COD_s consumption rate remained relatively stable from the first day till the end of the logarithmic growth phase, i.e. the day when COD_s consumption ended or significantly decelerated. As seen from the Figure 8.1, the COD_s consumption rate was more affected by the experimental arrangements and conditions than on the concentration of added glucose (less deviation inside particular experiment than between experiments).

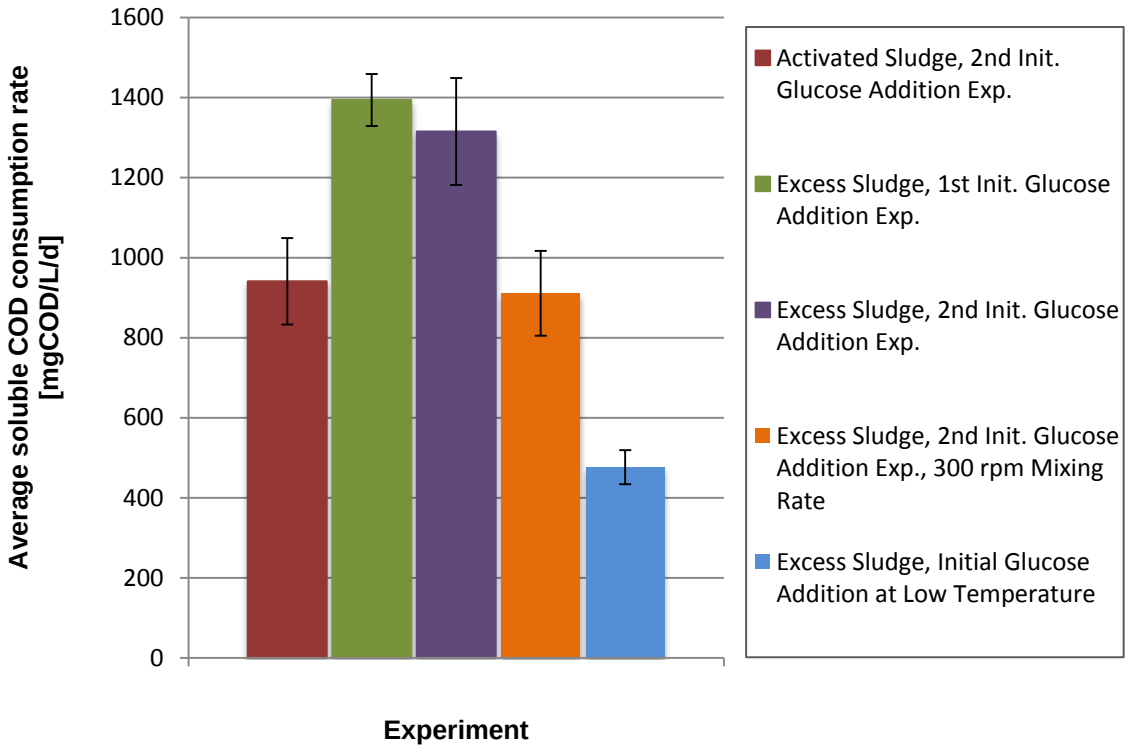

Figure 8.1. *The average COD_s consumption rates at the logarithmic growth phases.*

The average initial VSS concentrations were as presented in Figure 8.2. For the study, it would have been beneficial to use similar sludge in every experiment, but this was not possible due to poor preservability of the sludge. Since no external nitrogen was added, the concentration of nitrogen was mainly determined by the volume of sludge in the cultivation, i.e. experiments with higher initial VSS concentration had higher initial nitrogen concentration. The same initial glucose concentrations were utilized in every experiment, but the initial VSS was increased time after time. This resulted to lower C/N ratios compared to previous experiments.

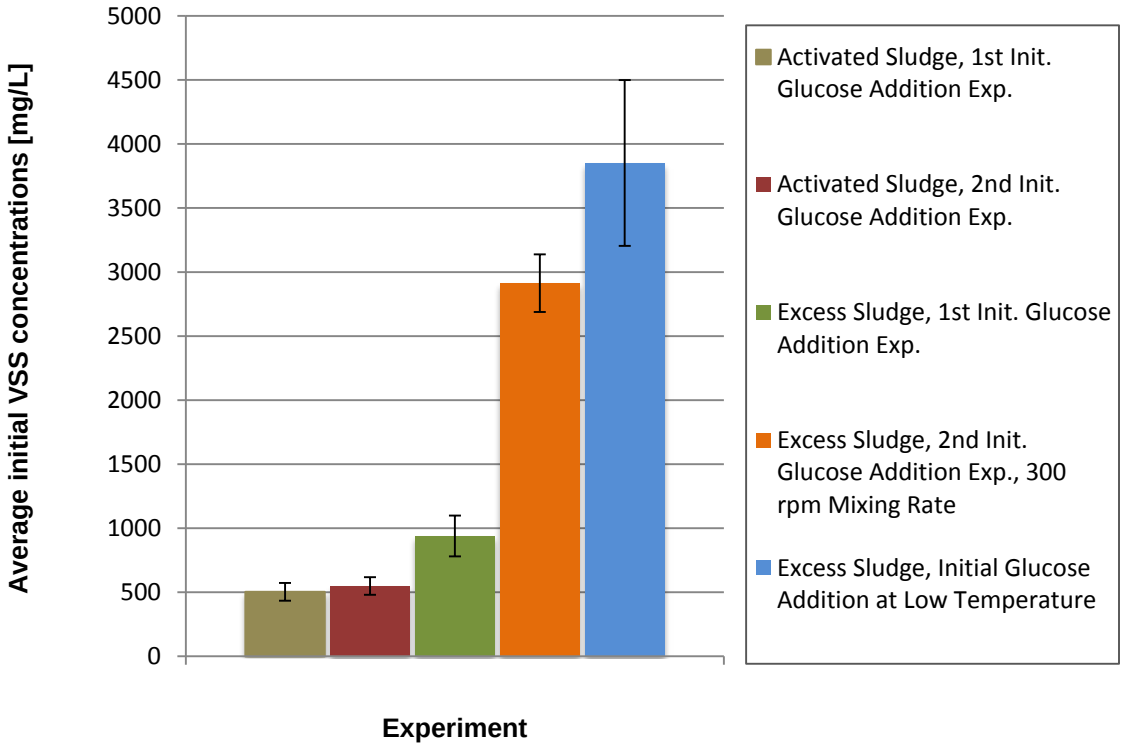

Figure 8.2. *The average initial VSS concentrations.*

In the 2nd excess sludge experiment, the higher mixing rate seemed to decelerate the COD_s consumption. However, 300 rpm mixing was still chosen to be used in the following experiments. It is assumed that the 2nd excess sludge experiment suffered from less microbiologically active sludge or some growth inhibiting substances in the batch bottles. As seen in the Results chapter, also the final biomass (Figure 7.20) and lipid (Figure 7.24) results were lower compared to the other experiments.

When Figure 8.1 and Figure 8.2 are compared it can be concluded that in addition to experimental arrangements (temperature, sludge quality and the concentration of available DO), concentration of active biomass seemed to be the most important factor affecting the substrate utilization rate.

The lowest COD_s consumption rate was detected in the experiment conducted at 5.6 ^oC. In theory, the growth rate is said to double with approximately every 10 ^oC increase (Metcalf & Eddy, Inc. 2003). The difference in temperature between low temperature experiment and other conducted experiments was 21.4 ^oC, meaning approximately 4 times higher growth rate. Substrate utilization rate and growth rate, however, are not exactly correlated, because substrate is also used for maintaining the living cells (Rittmann & McCarty 2001).

The maximum increases in VSS and the highest final VSS concentrations were as presented in Table 8.1. The 1st activated sludge initial glucose addition experiment is not comparable to the other experiments, since the cultivation was ended before the end of logarithmic growth. The highest increase in VSS concentration was achieved when C/N ratio was over 150:1. Results indicate that lower C/N ratio, i.e. higher N_{tot} concentration, resulted to lower VSS concentration. However, the 2nd excess sludge experiment may have suffered from poor sludge quality, so the indication is not completely trustworthy. This indication differs completely from Mondala et al. (2011) study, in which the initial C/N ratios were 10:1, 40:1 and 70:1, and initial glucose loadings were 20, 40 and 60 g/L. In Mondala et al. (2011), increasing glucose uptake rates and higher total biomass concentrations with decreasing C/N ratio were detected. Maximum biomass was achieved with 10:1 initial C/N ratio and 60 g/L glucose loading. However, initial glucose concentrations in Mondala et al. (2011) study are 2 - 30 times higher than in this study, resulting almost five times higher final VSS concentrations. In Mondala et al. (2011), higher final biomass concentration at 10:1 C/N ratio compared to 70:1 was primarily affected by higher initial concentration of NH_4-N added in the artificial wastewater culture medium. In this study, the N_{tot} concentration was proportional to the initial VSS concentration. Nitrogen was most likely present as organic nitrogen in the sludge biomass, meaning that it was not as easily utilized by microorganisms.

Table 8.1. *Maximum VSS increases of the batch bottle experiments.*

Experiment	Initial glucose addition	Initial C/N ratio	Initial VSS [mg/L]	Final VSS [mg/L]	VSS increase [mg/L]	Cultivation ended at
Activated sludge 1st initial glucose addition exp.	8 g/L	n.a.	560 ± 30	1 600 ± 70	1040 ± 100	Day 4, 3 950 mg/L COD_s left
Activated sludge 2nd initial glucose addition exp.	10 g/L_b	**155:1**	540	2 980	**2 440**	Day 9, 630 mg/L COD_s left. Log. growth ended 1 day before
Excess sludge 1st initial glucose addition exp.	10 g/L	151:1	1 170 ± 70	3 120 ± 430	1 950 ± 500	Day 6, 240 mg/L COD_s left. At the end of log. growth
Excess sludge 2nd initial glucose addition exp. 300 rpm mixing	8 g/L 300 rpm	59:1	3 070 ± 0	3 270 ± 50	200 ± 50	Day 7, 560 mg/L COD_s left. Log. growth ended 2 days before
Excess sludge Initial glucose addition at low temperature	10 g/L	n.a.	4 070	5 480	1 410	Day 25, 1 280 mg/L COD_s left. Log. growth ended 6 days before

n.a. = not analyzed

In addition to the assumed less microbiologically active sludge or growth inhibiting subjects in the 2nd excess sludge initial glucose addition experiment, the low final VSS concentrations can be explained according to Figure 4.1 and Figure 4.2. When external substrate (glucose) ran out, biomass was used as nutrition. (Karttunen et al. 2004.) In the low temperature experiment, VSS concentration in the bottle with 10 g/L initial glucose addition remained higher than the initial VSS even 6 days after logarithmic growth had ended (Figure 7.27), indicating that also the endogenous decay rate suffered from the temperature being below optimum. As a summary, it can be deduced from the results that at 27 ^{o}C endogenous decay and degradation of the biomass begin very soon after the soluble substrate runs out. Thus, when maximum biomass (or lipid) production is desired, the control of SRT and F/M ratio is vital for the success of the process.

In Mondala et al. (2011), some indications of substrate inhibition (high glucose residuals relative to microbial cell concentration) with 60 g/L initial glucose concentration and 70:1 C/N ratio were detected A lag phase was observed during the first 24 h of fermentation in all C/N ratios investigated, after which the duration of the logarithmic growth phases and the cell growth rates differed significantly. As discussed above, the COD_s consumption rates in this study remained quite similar in particular experiment, regardless of the C/N ratio. However, some indications of higher COD_s (glucose) residuals in result of 8 or 10 g/L initial glucose concentrations compared to glucose residuals in result of 2, 4 or 6 g/L initial glucose concentrations were detected, especially in the

2nd activated sludge initial glucose addition experiment and in the excess sludge initial glucose addition experiment at low temperature (Figure 7.4 and Figure 7.25).

8.1.2 pH

In Viinikanlahti WWTP alkalinity (lime) is added into wastewater before it enters the aeration basin, in order to maintain the process pH in the general optimum range of 6.5 - 7.5 (Rittmann & McCarty 2001; Sandelin 2010). In this study, the pH was not controlled in any of the batch bottle experiments. In every experiment, the initial pH was in the range of 6.5 - 8.0, but the pH changes during cultivations differed significantly in some cases, as discussed below.

In the 1st activated sludge initial glucose addition experiment, pH increased quite constantly from approximately 6.5 to 7.5 and even to 8.4 (Figure 7.3). In the 2nd activated sludge experiment, pH remained approximately between 7.0 and 8.0 in all but one of the bottles. In one replicate with 10 g/L initial glucose addition, pH sunk even below 3.0 and rose again near 8.0 in the end of the cultivation (Figure 7.8). A greenish colour, differing from the brownish colour of other cultivations was detected, as well as sweet odour. Although pH decrease did not affect the substrate utilization (Figure 7.4) or lipid production (Figure 7.10), a lower concentration of VSS was detected in the end of the cultivation (Figure 7.6). This is contradictory to the volumetric lipid concentration results and indicates an error in the VSS analysis or different filtration characteristics in the lower pH cultivation. Microscopic examination showed no differences between the cultivations.

The 1st excess sludge initial glucose addition experiment showed only slight pH increase from approximately 7.0 to 8.0 (Figure 7.15). However, in the 2nd excess sludge experiment, the pH decreased near 4.0 from the estimated initial pH of 7.5 in every bottle with initial glucose addition over 4 g/L, but rose again towards the end of the cultivation (Figure 7.22). In the initial glucose addition at low temperature experiment, pH remained again between 7.0 and 8.5 (Figure 7.28). As seen from Table 7.2, the initial N_{tot} concentration in the 2nd excess sludge experiment was over two times the N_{tot} concentration in the 1st excess sludge experiment. These results indicate that in the presence of organic substrate, the organic nitrogen bound to the sludge biomass was degraded into NH_4-N, which was then oxidized by nitrifying bacteria. Nitrification produces acidity and consumes alkalinity, which explains the decrease in pH. In 5.6 ^{o}C, nitrification is far less likely to occur. This explains the pH in the low temperature experiment. (Rittmann & McCarty 2001; Finland's Environmental Administration 2011.)

According to Mondala et al. (2011), the decrease in pH can have been caused by one or more of the following: excretion of endogenously produced organic acids by the activated sludge microorganisms; use of ammonium sulphate ($(NH_4)_2SO_4$) as the nitrogen source due to the preferential assimilation of NH_4^+ radical and release of acid in the culture; and the formation of carbonic acid due to the dissolution of the CO_2 produced by microorganisms into the culture.

8.1.3 Aeration and Mixing

Because overall lipid accumulation of aerobic microorganisms in the activated sludge was studied, the desired DO level in the batch bottle experiments was above 2 mg/L, which according to literature is usually enough to keep oxygen from being rate limiting (Rittmann & McCarty 2001). Oxygen was supplied to the batch cultivations by mixing the bottles with an orbital shaker. Generally, higher active biomass concentration and higher COD_s consumption rate resulted to lower DO concentration. As in all experiments, DO measurement was challenging because of solid particles tended to stick to the probe's head.

In the activated sludge experiments, 150 rpm mixing rate was used. In the 1st activated sludge experiment DO maintained below 3 mg/L for the whole cultivation period (Figure 7.3). In the 2nd activated sludge experiment, DO decreased below 2 mg/L in the bottles with 10 g/L initial glucose concentration (Figure 7.9), and may have caused the slightly lower COD_s consumption rate compared to bottles with 8 g/L initial glucose addition (Figure 7.5).

In the 1st excess sludge initial glucose addition experiment, 150 rpm mixing rate was used. The lowest measured DO concentration was 0.98 mg/L, although generally the DO concentrations remained above 2 mg/L (Figure 7.16). Quite large deviations were detected between parallel batch bottles. In the 2nd excess sludge initial glucose addition experiment, 300 rpm mixing rate was used for bottles with initial glucose addition of 8 and 10 g/L. Due to significantly higher concentration of active biomass (three times higher initial VSS concentration), DO went near 0 mg/L in every bottle with initial glucose addition and 150 rpm mixing rate (Figure 7.23). The 300 rpm mixing rate was enough to maintain the DO above 4.5 mg/L. In the initial glucose addition at low temperature experiment, lower cultivation temperature suppressed the biomass growth enough to keep the DO above 8 mg/L in every bottle (Figure 7.29).

8.1.4 Lipid accumulation

The maximum lipid contents and concentrations of the batch bottle experiments were as shown in Table 8.2. With initial external carbon (glucose) addition, the lipid content increased from initial in all four experiments except the initial glucose addition at low temperature. Increase in lipid concentration was detected in every experiment except the 2nd excess sludge initial glucose addition. Naturally, increased lipid concentration occurred partially due to increased biomass concentration, but the increased lipid content in the sludge (mass-% of dried sludge) indicates enhanced lipid accumulation under high carbon loading. The highest concentration increase compared to initial sludge lipids (from initial 20 mg/L to 147 mg/L at the end of the cultivation) was achieved in the 2nd activated sludge initial glucose addition experiment.

Table 8.2. *Maximum lipid contents and concentrations of the batch bottle experiments.*

Experiment	Initial glucose addition	Initial C/N ratio	Lipid content [mass-%]	Lipid conc. [mg/L]	Min/max. during cultivation		Lipid sample taken at
					pH	DO [mg/L]	
Act. sludge, 2nd exp.	10 g/L_a	89:1	*5.2**	*20**	2.8 / 7.9	1.8 / 7.2	Day 9, 630 mg/L COD_s left. Log. growth ended 1 day before
			7.4	147			
Exc. sludge 1st exp.	10 g/L	151:1	*6.3**	*129 ± 1**	7.0 / 7.7	2.6 / 7.9	Day 6, 240 mg/L COD_s left. At the end of log. growth
			8.4 ± 0.0	229 ± 38			
Exc. sludge 2nd exp. 300 rpm	4 g/L	30:1	*6.3**	*388 ± 3**	4.6 / 7.4	0.2 / 4.5	Day 3, 519 mg/L COD_s left. Log. growth ended 1 day before
			9.2 ± 0.6	290 ± 37			
Exc. sludge low temperature	10 g/L	n.a.	*8.6**	*516**	7.2 / 8.1	8.0 / 10.6	Day 21, 1 593 mg/L COD_s left. Log. growth ended 2 days before
			8.0	570			
			11.4	880 ± 66			

* Initial activated/excess sludge lipids
n.a. = not analyzed

Compared to the study by Mondala et al. (2011), the results are congruent to a certain extent. In Mondala et al. (2011), with an initial C/N ratio of 70:1 and glucose loading of 60 g/L, a maximum of 17.5 ± 3.9 mass-% lipid content was achieved after 7 days of cultivation. Raw activated sludge had average lipid content of 11.0 ± 1.7 mass-%. Volumetric lipid yields in Mondala et al. (2011), however, were much higher compared to ones in this study, due to higher glucose loading which led to higher biomass concentration. Maximum lipid concentration, approximately 2 250 mg/L, was achieved with initial C/N ratio of 40:1 and glucose concentration of 60 g/L.

According to Ratledge (2005), for lipid accumulation to occur, C/N ratio should be above 20:1. According to Mondala et al. (2011) low C/N ratio (10:1) favoured nonlipid biomass production, whereas a high C/N ratio (70:1) favoured lipid accumulation. However, in order to attain maximal lipid concentration, nonlipid biomass yield needs to be enhanced as well to provide sufficient cellular vehicles that contain the accumulated lipids (Mondala et al 2011). Comparing the effects of C/N ratio for lipid accumulation in this study was difficult because all the experiments differed from each other in many ways. The most influential parameters may have been the duration of the cultivation and the point of lipid sampling. The reason for lower lipid content and concentration compared to initial lipids in the two above-mentioned experiments might have been endogenous decay due to prolonged cultivation. Lipid sampling was done 1 or 2 days after logarithmic growth phase ended, which may have caused decrease in the achieved lipid content and concentration. Active biomass requires energy for cell maintenance, which includes cellular functions such as motility, repair and resynthesis, osmotic regulation transport and heat loss (Rittmann & McCarty 2001). In absence of glucose, intra-

cellular lipids were used as energy and carbon source for maintenance. Also Mondala et al. (2011) reported a slight decrease in lipid concentration after glucose had ran out, in activated sludge cultivated at 60 g/L initial glucose loading and initial C/N ratio of 10:1.

It has been previously reported that culture pH values below 3 reduced biomass and lipid production in pure cultures of oleaginous yeasts and bacteria (Wayman et al. 1984; Davies 1988). In Mondala et al. (2011), lipid accumulation occurred also at low pH levels, suggesting that some members of wastewater microbial communities are capable of accumulating lipids at these extreme conditions. In this study, pH during the cultivations remained above 4.5 except in one experiment, in which no significant effect on lipid accumulation were detected. Likewise, there were no clear signs on the effects of DO concentration on lipid accumulation, although DO levels below 2 mg/L may have slowed down the growth in some experiments. In low temperature experiment, higher COD_s (glucose) residuals than in the other experiments remained in the culture after logarithmic growth phase ended, indicating that microbes were not able to utilize as much of the substrate than at higher temperature. Thus, also lipid accumulation may have suffered.

8.2 Laboratory Scale Activated Sludge Reactor

8.2.1 1st Reactor Run

During the whole reactor run, the process suffered from poor sludge settling properties. Pinpoint flocs and dispersed growth were detected. Naturally these problems lead to washout of the solid particles, decreased treatment efficiency and decreasing MLSS concentration in the aeration tank. In the beginning of the run, MLSS concentration of the activated sludge from Viinikanlahti WWTP was approximately 6 100 mg/L. During the first ten days of the reactor run, the MLSS decreased by approximately 1 000 mg/L (Figure 7.31). Also pH in the aeration tank decreased from 6.8 to 5.9 during the first ten days of the run (Figure 7.34). One of the reasons for poor sludge settling properties may have been the pH decreasing out of optimal range, which has caused decelerated growth and poor floc formation. The decreased pH also indicates that nitrification occurred (Sherrard 1976; Rittmann & McCarty 2001). The MLSS concentration increased 600 mg/L from day 10 to day 14, while COD_s concentration decreased approximately 128 mg/L on the same time period (Figure 7.32). This may have been due to slightly improved scraper modification. From day 14, the MLSS decreased again.

The DO concentration remained above 5.5 mg/L for the whole reactor run. When a 2 mg/L DO concentration is desired to keep oxygen from being rate limiting, the aeration may have been slightly too efficient. Too intense aeration may deteriorate the sludge flocs, and at least it is not economically reasonable (Rittmann & McCarty 2001; Holenda et al. 2008). However, because so many other factors might have affected the poor treatment results, aeration was decided to continue with the same flow rate also in the 2nd reactor run.

One of the major problems during the 1st reactor run was the biological degradation and settling of the solid particles in the wastewater canisters, which significantly affected the organic load of the reactor. Because of premature degradation, the influent COD_s on day 10 was almost 40% lower than the one used in operation parameter calculations (110 mg/L), causing approximately the same decrease to occur in the F/M ratio. From day 14, the influent CODs concentration was achieved to maintain near the desired level. Reason for the constant decrease of influent COD_{tot} from between days 10 and 18 (Figure 7.33) was the rapid settling of the solids into the bottom of canister, while influent was pumped to the reactor approximately 10 cm above the canisters base.

As seen from the Figure 3.2, low F/M ratio and high SRT lead to pinpoint floc formation and weaken the sludge settling characteristics. During the 1st reactor run, waste sludge was not removed at all. When all the sludge, both dead and living cells, remains in the system, the SRT increases and settling problems occur (Rittmann & McCarty 2001; Metcalf & Eddy, Inc. 2003.) In 20 days, the process failed totally, and only 2.5% COD_{tot} reduction was obtained, when sludge did not settle at all and washed out from the settling tank. The 1st reactor run was ended. Useful insight was gained for the 2nd run.

8.2.2 2nd Reactor Run

The 2nd run with laboratory scale activated sludge reactor continued for 87 days. Between days 30 and 48, the reactor was run with conventional loading and normal maintenance but no control analyses were conducted. In order to prevent the early biodegradation of the influent wastewater, wastewater canisters were stored in the refrigerator at 4 ^{o}C. As seen from Figure 7.36, before introducing the reactor to higher organic loads, the influent COD_s maintained between 100 and 150 mg/L without few exceptions. The average COD and MLSS concentrations during the 2nd reactor run were as presented in Table 8.3. On average, the influent COD_s concentration before glucose addition was 117 ± 12 mg/L.

Table 8.3. *The average COD and MLSS concentrations during the 2nd reactor run.*

	Average	Influent	Effluent	Activated sludge
Days 0 - 59	COD_s [mg/L]	117 ± 12	41 ± 10	
	COD_s Reduction [%]	66 ± 7		
	COD_{tot} [mg/L]	219 ± 33	56 ± 13	
	COD_{tot} Reduction [%]	74 ± 6		
	MLSS [mg/L]			3 430 ± 980
Days 60 - 73	COD_s [mg/L]	218 ± 27	46 ± 4	
	COD_s Reduction [%]	79 ± 3		
	COD_{tot} [mg/L]	331 ± 73	62 ± 6	
	COD_{tot} Reduction [%]	81 ± 4		
	MLSS [mg/L]			1 850 ± 160
Days 74 - 78	COD_s [mg/L]	311	48	
	COD_s Reduction [%]	85		
	COD_{tot} [mg/L]	415	60	
	COD_{tot} Reduction [%]	86		
	MLSS [mg/L]			1 990 ± 160
Days 79 - 87	COD_s [mg/L]	426 ± 20	84 ± 17	
	COD_s Reduction [%]	80 ± 4		
	COD_{tot} [mg/L]	560 ± 34	104 ± 19	
	COD_{tot} Reduction [%]	81 ± 4		
	MLSS [mg/L]			2 330 ± 240

As seen from Table 8.3, the reactor as well as the influent remained relatively stable during the 2nd run. Inside the presented time periods, there are quite small deviations in the COD concentration and reduction-%. For comparison, the analyzed COD_s and COD_{tot} reduction-% from Viinikanlahti WWTP were 68% and 83%, respectively. Also the BOD results (Table 7.3) indicate that as far as the treatment efficiency is considered, the 2nd reactor run was successful.

In order to maintain alkalinity and preferred pH, calcium oxide (CaO) was added into the influent canister, which prevented the activated sludge pH from decreasing (Figure 7.38). The DO concentration remained above 4.0 mg/L for the whole run. After day 65, sludge's DO concentration according to the measurements was higher than during the first 29 days of measurement, which may have been due to lower MLSS concentration (Figure 7.35).

The main problem during the 2nd reactor run was the decreasing MLSS concentration. The initial MLSS concentration was 4 990 ± 80 mg/L. On day 69 the MLSS was at the lowest, 1 640 ± 10 mg/L, although influent COD_s concentration had been almost twice the initial since day 59 (Figure 7.35). Perhaps due to relatively short stabilization time, the MLSS did not recover even when 4 times higher organic loading than the initial was utilized. The significant MLSS decrease was mainly due to high sludge removal rate. During the 2nd reactor run the sludge settling problem was partially solved by removing the excess sludge from the aeration tank by a pump. Due to pump modification, the minimum sludge removal rate achieved was 750 mL/d, which caused the SRT to be

only 15.2 d compared to approximately 30 d in Viinikanlahti WWTP. Biomass simply could not grow as fast as the sludge was removed from the aeration tank.

Nitrification-% according the analysis conducted during the 2nd reactor run was 88%. According to Sandelin (2010), nitrification-% in Viinikanlahti WWTP is 99.9%. Nitrifying bacteria are slow growers that can exist in the sludge only with long SRT (Rittmann & McCarty 2001; Clara et al. 2005), so halving the SRT may have had an influence on the nitrification.

The lipid accumulation was also cursorily studied during the 2nd reactor run. Although the organic loading was increased to almost 4 times the initial, the concentration of glucose was still very minor compared to the batch bottle experiments or to the study by Mondala et al. (2011). As seen from the Table 7.4, higher organic loadings produced slightly more lipids in mass-%, but the mg/L sludge lipid concentration was lower than initial because of the lower MLSS of the laboratory scale reactor.

9 CONCLUSIONS

- The lipid content in sludge can be increased by addition of external carbon sources. However, large concentrations of carbon are required which would significantly decrease the cost-efficiency and independency of the process.

- The highest lipid content (9.2 mass-% compared to initial 6.3 mass-%) was achieved with addition of 4 g/L glucose. The highest lipid concentration (570 mg/L compared to initial 516 mg/L) was achieved with addition of 10 g/L glucose. In addition, over 100 mg/L increases in lipid concentration were detected with initial glucose addition of 10 g/L in both activated sludge and excess sludge experiments.

- Duration of the cultivation and the point of sampling have a large effect on biomass and lipid concentration. Thus, when maximum biomass or lipid production is desired, the control of SRT is vital for the success of the process. Sludge needs to be utilized when it is in the logarithmic growth phase.

- Further batch bottle experiments should include: 1) studying the kinetics of lipid accumulation by taking samples during the cultivation; 2) controlling the cultivation pH, DO and temperature in order to find out the optimum conditions for activated sludge oleaginous microbes and 3) studying the microbial composition in activated sludge exposed to high carbon loading.

- As far as the treatment efficiency is considered, the 2nd reactor run was successful. Main problem was the decreasing MLSS concentration. More maintenance and readjustment of the process parameters is required while the reactor is running.

- With higher organic loadings, slightly higher lipid content (mass-% of dried sludge) was achieved in the laboratory scale reactor compared to the initial activated sludge. Sludge's lipid concentration (mg/L) was lower because of the decreased concentration of suspended solids.

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