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Disintegration and Degradation of Fish Feed Pellets and Feces Under Aerobic Marine Conditions

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Summary:

Fish farming industry is a major food industry which cover significant parts of the nutritional requirement of the world's population. Due to nutritional enrichment by uneaten pellets and fish feces, negative environmental impacts such as eutrophication, saprobiation and hypoxia condition in benthic zone occur in semi-enclosed fjord system.

As the main aim of this study the degree and kinetics aerobic degradation of four sizes of fish pellets (P 1000, P 500, P 200, P 80) and fish waste were determined using static (OxiTop) and dynamic (Micro-Oxymax) respirometry. Chemical analysis of fish pellet solution and fish waste before and after BOD tests were carried out to determine the nutrition release after aerobic degradation. Sinking velocity of fish pellet, dissolution of nutrient and disaggregation rate of pellets were determined to check the influence from these processes for the aerobic degradation.

The TN:TP ratios after BOD test of 28 days and 12 days for pellets is about 3:1 and 3:2 respectively. This suggest the ratio of nutrient supply to marine system after fully and partially aerobic degradation of fish feed. The 50% of final oxygen consumption ($t_{1/2}$) for P 1000 and P 500 is about 12 days while its 6 days for P 200 and P 80. The degree of aerobic degradation varies as P 200> P 1000, P 500, about 75%-80% and for P 80, about 70%. This is varying with the physical condition of the pellet as whole, grinded, and autoclaved. The degradation kinetics depend on the initial size of pellet, P 200>P 80>P 500>P 1000 demonstrated by the size dependent pseudo first order degradation coefficient (0.09 d^{-1}>0.08 d^{-1}>0.0003 d^{-1}>0.0001 d^{-1}) and kinetics vary with physical condition of pellet as pellet grinded autoclaved>pellet grinded> whole pellet for P 200 due to stimulate hydrolysis of complex compounds in pellet with heat, increase substrate surface area and dissolution of pellets. k_1 value for fish waste is about 0.19 d^{-1} at partially biodegradation stage after half completion of the BOD test. Settling velocity of the pellets where found to be 0.11, 0.10, 0.08 for P 1000, P 200, and P 500, respectively. P 1000 and P 500 pellet disaggregate more during sinking and normally take more time to fully dissolution due to fat layer formation. Analyzing all results from each pellet type using above experiments P 200 (higher kinetics, higher degree of aerobic biodegradation, less amount of organic matter content and dissolution without fat layer formation) make less contribution to negative environmental effect than bigger pellets like P 1000, P 500 (lower k_1 value, higher disaggregation, less dissolution) in marine fjord water system.

Preface

This report presents the theoretical and experimental results of disintegration and degradation of uneaten fish pellets and fish sludge including basically fish feces under aerobic marine condition. Further negative environmental effect as eutrophication, saprobiation comes with fish waste are explained using biological oxygen demand tests, using chemical analysis, using physical disaggregation and dissolution rate, and using settling velocity of pellets. This work was executed as the 4th semester master thesis of Master of Science of Energy and Environmental Technology (EET) at University of South-Eastern Norway (USN). This work was performed at University of Stavanger (UiS) under supervision of Prof. Roald Kommedal and Prof. Rune Bakke.

First and foremost, I would like to express my utmost gratitude to Prof. Rune Bakke and Prof. Roald Kommedal for their significant input and supervision during this study to successfully complete this work. I also like to convey my gratitude to Prof. Lars Andre Tokheim for giving me an opportunity to do my thesis work at UiS and Associate Prof. Carlos.Dinamarca for supporting with chemical analysis equipment.

I must specially thank to Prof. Roald Kommedal (Co- supervisor) for guiding me with his great knowledge and to laboratory staff in Faculty of Science and Technology at UiS for supporting me with the laboratory experiments.

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Sandnes, Norway 25/04/2018

Anupama Wathsalani Pupulawaththa

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Nomenclature

Roman Symbols

Symbol	Description	Units
$\gamma_{Protein}$	Specific theoretical oxygen demand for protein	$\frac{g_{O_2}}{g_{Protein}}$
γ_{Fat}	Specific theoretical oxygen demand for fat	$\frac{g_{O_2}}{g_{Fat}}$
$\gamma_{Carbohydrates}$	Specific theoretical oxygen demand for carbohydrate	$\frac{g_{O_2}}{g_{Carbohydrates}}$
$\Delta p(O_2)$	Difference in partial oxygen pressure	hPa
μ	Dynamic viscosity of sea fluid	$kgm^{-1}s^{-1}$
$\mu_{sea\ water}$	Dynamic viscosity of sea water	$kgm^{-1}s^{-1}$
ρ_a	Density of the fluid	kgm^{-3}
ρ_P	Density of the particle	kgm^{-3}
$\rho_{sea\ water}$	Density of sea water	kgm^{-3}
$BOD_{Initial}$	Biological oxygen demand value at the beginning	mgL^{-1}
BOD_{Final}	Biological oxygen demand value at the end	mgL^{-1}
$BOD\ (t)$	Biological oxygen demand value at a given time	mgL^{-1}
d	diameter of the particle	m
g	gravitational acceleration	ms^{-2}
k_1	Pseudo first order degradation coefficient, first order reaction rate constant	$days^{-1}$
$\mathrm{m(C)}$	mass of carbon	mg
$\mathrm{m}(O_2)$	oxygen consumption	mg
m_P	Mass of particle	kg

SCOD	Soluble chemical oxygen demand	mgL^{-1}
t	Time	$days$
TCOD	Total chemical oxygen demand	mgL^{-1}
ThOD	Theoretical oxygen demand	g_{O_2}
TN	Total nitrogen	mgL^{-1}
TP	Total phosphorous	mgL^{-1}
V_{tot}	Bottle volume	mL
$W_{volatile,1}$	Weight of residue and dish after ignition	mg
$W_{volatile,2}$	Weight of retained solid matters with filter paper after ignition	mg
$W_{total,1}$	Weight of dried residue and dish	mg
$W_{total,2}$	Weight of dried retained sample with filter paper	mg
$W_{sample,1}$	Weight of wet sample and dish	mg
$W_{sample,2}$	Weight of retained wet sample with filter paper	mg
W_{dish}	Weight of dish	mg
W_{FP}	Weight of filter paper	mg

Constants

Symbol	Description	Values	Units
$\propto$	Bunsen absorption coefficient	0.03103	-
$M(C)$	Molecular weight of carbon	12.01	g/mol
$M(O_2)$	Molar weight of oxygen	32	$gmol^{-1}$
R	Gas constant	83.144	$LhPamol^{-1}K^{-1}$
T_m	Measuring temperature	293.15	K
T_0	Absolute zero temperature	273.15	K

1 Introduction

Fish industry is important industry in the world fulfilling nutrition requirement of people providing long chain omega 3 fats, iodine, vitamin D and calcium (Bannister et al., 2016). Currently, more than half of the fish is used for making food around the world. From total aquaculture farming, fin fish farming is about 68% (Subasinghe, 2017). Salmon fish farming supply about 1.3 million tons per year in Norway and expected to increase the annual production from 5 million tons. With these expansions the waste generation also increase with uneaten pellets, feed, nutrients and other chemicals (Chen et al., 1999, Piedecausa et al., 2009, Bannister et al., 2016, Vassallo et al., 2006, Kutti et al., 2007).

With the nutritional enrichment and particle matter addition with aquaculture waste negative impact course for the environment as excess growth of plant and algae on the water (eutrophication), hypoxia condition affecting benthic environment, making environment toxic for fish increasing ammonia level, odor generation and so on (Merceron et al., 2002, Kuokkanen et al., 2004, Pérez et al., 2014, Kutti et al., 2007, Chen et al., 1999, Piedecausa et al., 2009, Vassallo et al., 2006, Bannister et al., 2016, Klanjšček et al., 2012).

By reviewing literature get an idea about the environmental effect of aquaculture system comes with organic matter depletion with aerobic degradation, settling velocity of feed and feces, and models. Further currently available solutions to reduce the environmental effect at feed manufacturing industrial level as by taking action to avoid the disintegration of feed pellets at the production level by changing formulation and changing way of manufacturing (Yandi and Kurtoğlu, 2016), and at the fish farms using benthic species, monitoring system, particle removing system and so on (Wang et al., 2013). By using these methods can be able to supply high nutritious food for the fish and reduce environmental damage.

The aerobic degradation of organic compounds come with fish feces and feed in fjord system study using OxiTop method and Micro-Oxymax methods, and further effect of settling velocity of fish pellets and feces and disaggregation rate of pellets are studying at laboratory level in this study. Eventually present study attempted to evaluate and try to figure out environmental effect comes from each of these study parts.

1.1 Objectives of the study

1.1.1 Main objective

Determine the kinetics and degree of aerobic degradation in marine fjord water of typical fish feed pellets and farmed salmon feces

1.1.2 Secondary objectives

- ✓ Chemical analysis of fish feed and feces before and after aerobic degradation
- ✓ Determine the settling velocity of fish pellets
- ✓ Determine the physical characteristics of pellets
- ✓ Determine the dissolution rate of the pellets

2 Literature Review

Current findings about fish feed and fecal composition, the environmental damage with aquaculture, available actions to reduce the damage, findings with feed and feces settling velocity, effect of settling velocity for environmental effect, available model regarding aquaculture system, about Biological oxygen demand(BOD), about chemical oxygen demand (COD), about other analysis used for determine the organic matter degradation, and determine the nutrient leaching of fish feed and feces in sea water are reviewed in this section.

2.1 Feed and fecal proximate composition

Fish body contains about 65% of protein. Protein is used for energy production more than other animals. Therefore fish need more protein than other mammals (Crab et al., 2005).

The proximate analysis of different fish feed varieties carried out by (Piedecausa et al., 2009, Ayuba and Iorkohol, 2013, Vassallo et al., 2006, Reid et al., 2009) are given in Table 2-1.

Table 2-1: Proximate composition of fish feed as percentage

	(Ayuba and Iorkohol, 2013), (Piedecausa et al., 2009)			(Vassallo et al., 2006)	(Reid et al., 2009)
	Type 1	**Type 2**	**Type 3**	**Type 4**	**Type 5**
Moisture	8.10 ± 0.27	8.62 ± 0.09	6.80 ± 0.06	-	8
Ash	9.44 ± 0.12	5.33 ± 0.02	7.30 ± 0.05	10.6	6.8
Crude protein	43.75 ± 0.00	52.65 ± 0.10	48.30 ± 0.52	45	40
Crude fat	11.93 ± 0.13	14.77 ± 0.05	21.10 ± 0.23	17	11.3

(Reid et al., 2009) has given a table with composition of feed with digestibility. Given that in Table 2-2 composition of feces is summarizing as follows.

Table 2-2: Proximate composition of fish feces as percentage

Content	**Feces composition (%)**
Protein	26.00
Fat	11.33
Carbohydrate	36.00
Mineral	13.33

From the nitrogen content if feed, can get an idea about the protein content of feed. Studies shows lipid content in dry weight of fish is higher than 1980s. Now it is about 30%-40% (Wang et al., 2013).

In the fish feed phosphorous digestibility of plant origin fish feed is lesser due to lack of phytase enzyme in fish body and produce particle wastes. But Protein digestibility is about 85 % and release high amount of nitrogen and comparatively carbon digestibility is also about 80% in fish body (Cheshuk et al., 2003, Mente et al., 2006, Olsen et al., 2008).

Digestibility of phosphorous in fish feed is about 50% and the percentage in feed is about 1.2% in Atlantic salmon fish feed. Digest percentage of phosphorous is about 0.6 % and 0.6% is in feces (Reid et al., 2009) in Atlantic salmon fish farm.

Using fish oil and meal for producing feed is one reason for increasing waste in aquaculture system (Crab et al., 2005).

2.2 Correlation between feed and fecal compositions

The digestion of fish is low and higher portion of feed is not digesting and remove via feces. The length of gut is small in fish and the ratio to body length is smaller. When consider about human intestine, it is about 2 times longer than body (Crab et al., 2005). The chemical content of fish feed and feces have a direct relationship as every other animal (Wang et al., 2013). has concluded after digestion of fish feed 40 % of carbon, 40 % of nitrogen and 25 % of phosphorous are contained in the fish body mass while about 40 % of carbon is used for the respiration process as carbon dioxide. Therefore, more than half percentage of carbon (60 %), nitrogen (60 %) and phosphorous (75 %) excreted in to the water bodies as feces same as 2009 Norwegian salmon aquaculture estimation. Yearly estimated amount is about 400 kt carbon, 50 kt nitrogen and about 10 kt of phosphorous is released as wastes from fish body (Wang et al., 2012, Wang et al., 2013). Because of high protein metabolism of fish ammonia release is high from fish body (Crab et al., 2005).

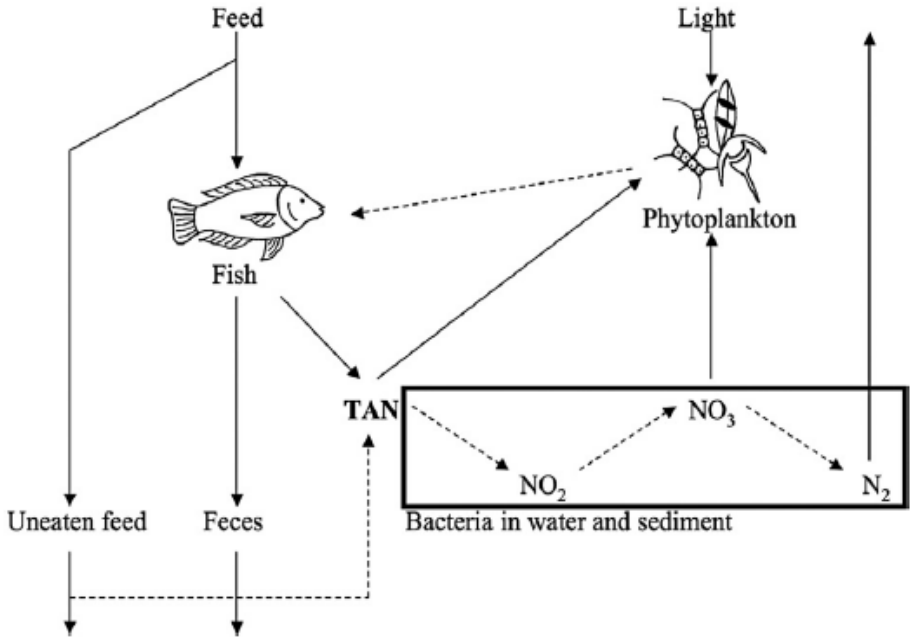

Figure 2-1: Nitrogen cycle in aquaculture pond (Crab et al., 2005)

Table 2-3: Animal and plant oil sources used commonly for fish feed formulation (FAO, 2018)

Fatty acid	Chemical structure	Name	Anchovy	Herring	Capelin	Menhaden	Redfish	Poultry fat	Canola	Soybean
14:00	CH3(CH2)12COOH	Myristic acid	7.4	6.4	7.9	7.3	4.9	0.9		0.1
16:00	CH3(CH2)14COOH	Palmitic acid	17.4	12.7	11.1	19	13.2	21.6	3.1	10.3
16:1n-7	CH3(CH2)5CH=CH(CH2)7COOH	Palmitoleic acid	10.5	8.8	11.1	9	13.2	5.7		0.2
18:00	CH3(CH2)16COOH	Stearic acid	4	0.9	1	4.2	2.2	6	1.5	3.8
18:1n-9	CH3(CH2)7CH=CH(CH2)7COOH	Oleic acid	11.6	12.7	17	13.2	13.3	37.3	60	22.8
18:2n-6	CH3(CH2)4CH=CHCH2CH=CH(CH2)7COOH	Linoleic acid	1.2	1.1	1.7	1.3	0.9	19.5	20.2	51
18:3n-3	CH3CH2CH=CHCH2CH=CHCH2CH=CH(CH2)7 COOH	α-Linnoleic acid	0.8	0.6	0.4	0.3	0.5	1	12	6.8
18:4n-3	-	-	3	1.7	2.1	2.8	1.1	1.1		
20:1n-9	-	-	1.6	14.1	18.9	2	17.2	0.1	1.3	0.2
20:4n-6	CH3(CH2)4CH=CHCH2CH=CHCH2CH=CHCH2 CH=CH(CH2)3COOH	Arachidonic acid	0.1	0.3	0.1	0.2	0.3	-	-	-
20:5n-3	CH3CH2CH=CHCH2CH=CHCH2CH=CHCH2CH =CHCH2CH=CH(CH2)3COOH	Eicosapentaenoic acid	17	8.4	4.6	11	8	-	-	-
22:1n-11	-	-	1.2	20.8	14.7	0.6	18.9	-	1	-
22:5n-3	-	-	1.6	0.8	0.3	1.9	0.6	-	-	-
22:6n-3	CH3CH2CH=CHCH2CH=CHCH2CH=CHCH2CH =CHCH2CH=CHCH2CH=CH(CH2)2COOH	Docosahexanoic acid	8.8	4.9	3	9.1	8.9	-	-	-
Σ n-6	-	-	1.3	1.4	1.8	1.5	1.2	19.6	20.2	51
Σ n-3	-	-	31.2	17.8	12.2	25.1	19.1	1	12	6.8

When considering nitrogen cycle in pond the nitrogen comes with feed. After feed intake by fish some of organic matter with nitrogen convert into biomass and other part remove from the fish body as ammonia or with feces. And ammonia come from uneaten pellets as well (Merceron et al., 2002, Wang et al., 2013, Kutti et al., 2007, Chen et al., 1999, Piedecausa et al., 2009, Pérez et al., 2014, Bannister et al., 2016, Aure and Stigebrandt, 1990, Gowen, 1994, L Carroll et al., 2003, Stigebrandt et al., 2004).

The Total Ammonia Nitrogen (TAN) transfers into nitrite and further into nitrate (by nitrification process by bacteria) and eventually into nitrogen gas (denitrification by bacteria) (Kuokkanen et al., 2004). Nitrogen gas formation is very little in aquaculture system. Phytoplankton species takes TAN and nitrate. If there is not enough bacteria to do the nitrification process increase the ammonia in the system providing toxic effect for the fish and other living organisms and make order generation (Merceron et al., 2002).

Because of unionized ammonia toxicity (> 1.5 mg/N/L), the level for commercial fish culture is about 0.025 mg/ N/L depend on the fish type. Ratio between nitrogen and carbon is low in salmon feces than in fish feed due to high protein retention rate of fish.

Even it is general thing to increase the chemical component content with increase fish body mass, only carbon content in fish feces has a direct relationship with fish weight (Wang et al., 2013).

More than 50 % of carbons content, half of nitrogen and very high percent of phosphorous in fish feed are included in fish feces after digestion. Phosphorous content in fish feces of Atlantic salmon (Chen et al., 1999, Kristiansen and Hessen, 1992)and silver perch (Kibria et al., 1997)are very high due to lack of digestibility. Therefore main phosphorous removal is occurred with feces (Wang et al., 2013, Kibria et al., 1997)

Lipid content, EPA, DHA in feces is lower due to high digestibility in Salmon fish. Some phytoplankton species and fish feces in salmon aquaculture sites shows similar lipid as omega -3 fatty acids. Uneaten fish pellets and feces supply food for other species as blue mussels and seas cucumbers in salmon fish cultivated areas (Wang et al., 2013).

Table 2-4shows range of chemical content and mean chemical content in feed and feces samples. In the feces dry matter content is vary from 10% to 30 % in wet basis. (Wang et al., 2013) says the amount of chemical composition in waste is not depend on the time and feed variety.

Table 2-4: Range of chemical content and mean chemical content in feed and feces samples (Wang et al., 2013), (Reid et al., 2009)

Chemical Component	Content in fish feed	Content in fish feces
Total carbon	540 ± 4	321 ± 32.1
Total nitrogen	58 ± 1	34.2 ± 3.4
Phosphorous	8.8 ± 0.5	23 ± 1.3

2.3 Environmental Impact

The inorganic nutrient content output of marine aquaculture system is very high due to uneaten feed, fish feces, branchial excretion, medical wastes and pesticides cause negative damage to the ecological system as increase eutofication (Merceron et al., 2002, Wang et al., 2013, Aure and Stigebrandt, 1990, Gowen, 1994, L Carroll et al., 2003, Stigebrandt et al., 2004, Kutti et al., 2007, Chen et al., 1999, Piedecausa et al., 2009, Vassallo et al., 2006, Pérez et al., 2014, Bannister et al., 2016).

The waste of nutrient occurs directly from fish feed and indirectly by fish. Pellets need to stable in water with less nutrient leach and maintaining the physical characters of the pellets as shape and size. When the stability of pellets low, in sea water pellets disaggregate into particles easily and leach nutrient easily with disaggregation (Yandi and Kurtoğlu, 2016). Now a days lots of large rivers, inland reservoirs, wetlands and seas affected by this eutrofication process(Istvánovics, 2010). Content of the fish feces as metal content further damage to the environment (Merceron et al., 2002, Uotila, 1991).

Fish feces and uneaten fish pellets containing nutrition content, are deposited in the sea bottom and create local environmental impact in salmon cage aquaculture in Norway and also in the world .Organic matter supply to the benthic environment mostly occur with uneaten fish feed and feces (L Carroll et al., 2003, Chen et al., 1999, Piedecausa et al., 2009). Most of the particle waste (Particulate organic carbon (POC), Particulate organic nitrogen (PON), Particulate organic phosphorous (POP)) in fish farms supply by uneaten fish particles (see Table 2-3). According to the estimation about 350 Kg/g of fish feed loss in salmon farms in 1980 (Piedecausa et al., 2009).

Laboratory studies suggest that 30% (about 350 Kg/g) of feed in dry weight is excreted as feces in salmon fish farm. This loss as feces in Mediterranean fish farm is about 350 g/kg fish produced (Piedecausa et al., 2009).

When consider the size of the fish feces pellets with the nutrient leaching, the smaller size have high surface area: volume ratio and leach higher amount of nutrients than big feces pellets (Chen et al., 1999).

Fish feces becomes the most problem occurring thing than fish feed for eutrophication these days. The Nitrogen carbon and phosphorous ratios in salmon feces (around 70 μg Nmg^{-1}°C and 1 N mg^{-1} P) comparable with some phytoplankton species (Wang et al., 2013) and lipid content (about 70 mgg^{-1} dry weight) as DHA, EPA is comparable with some diatoms causes spring bloom in Trondheim fjord (Sakshaug and Myklestad, 1973, Liang et al., 2003, Reitan et al., 1994, Renaud et al., 1994). The N excretion and P excretion rate are about 25 mg N/m2/d and about 7.17 mg $N/m^2/d$ in aquaculture system (Vanni, 2002). Water with high amount of dissolved P and N (DOC, DON) enhance the plant growth and break the balance of aquaculture system. Some experiments have succeeded by 50% reduction of phosphorous content in lakes to check phytoplankton growth. But if the eutrofication is higher the larger algae varieties come into the aquaculture system with small varieties which is difficult to control by limiting nutrients in water (Istvánovics, 2010).

The severity of the environmental damage depends on the amount of the nutrient, area and natural flushing capacity of the water body. With the slow current in swallow water, solid waste accumulated in the discharge point in the farm and further increase accumulation in the bottom (L Carroll et al., 2003).

Therefore, the environmental damage determine by assimilation capacity related to depth of the water body, topography and wave pattern and also damage depend on the amount of nutrient

from the waste the aquaculture system (FAO, 1986, Black, 2001, Gowen and Bradbury, 1987, Iwama, 1991, Wu, 1995).

As example high ammonia accumulation can be seen in salmon fish farm due to lack of capacity to waste removal (Merceron et al., 2002). Waste generation is related to amount of fish availability and feeding rates Michael,2003.

Figure 2-1 shows a summary for the nutrients come from the fish, fish feed and feces in salmon fish farms (Reid et al., 2009).

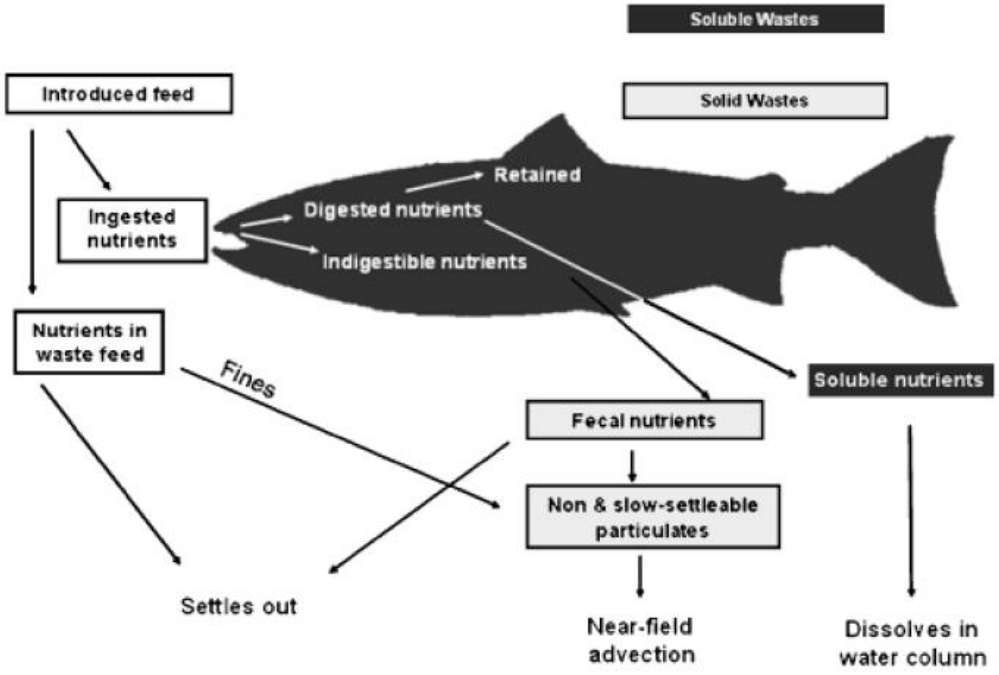

Figure 2-2: Fate of nutrients comes from fish feed in aquaculture farm

With aerobic degradation of this high amount organic matter waste hypoxia condition (lesser than 3 mg O2 L-1 for 50% benthic fauna) can occurs in benthic environment negatively affecting benthic species. This hypoxia condition becomes worst with eutrophication and saprobiation (Belfiore et al., 2003). And without acting to eutrophication and saprobiation, can create odor generation with anaerobic digestion in ponds. The damage is high in semi-enclosed sill fjord having still water. The amount of oxygen usage depend on the organic matter composition of fish feed (Klanjšček et al., 2012).

Due to lack of national monitoring system, less data availability, lack of sample representation, lack of covering of fish farm management system, it is hard to determine the level of environmental damage accurately (L Carroll et al., 2003).

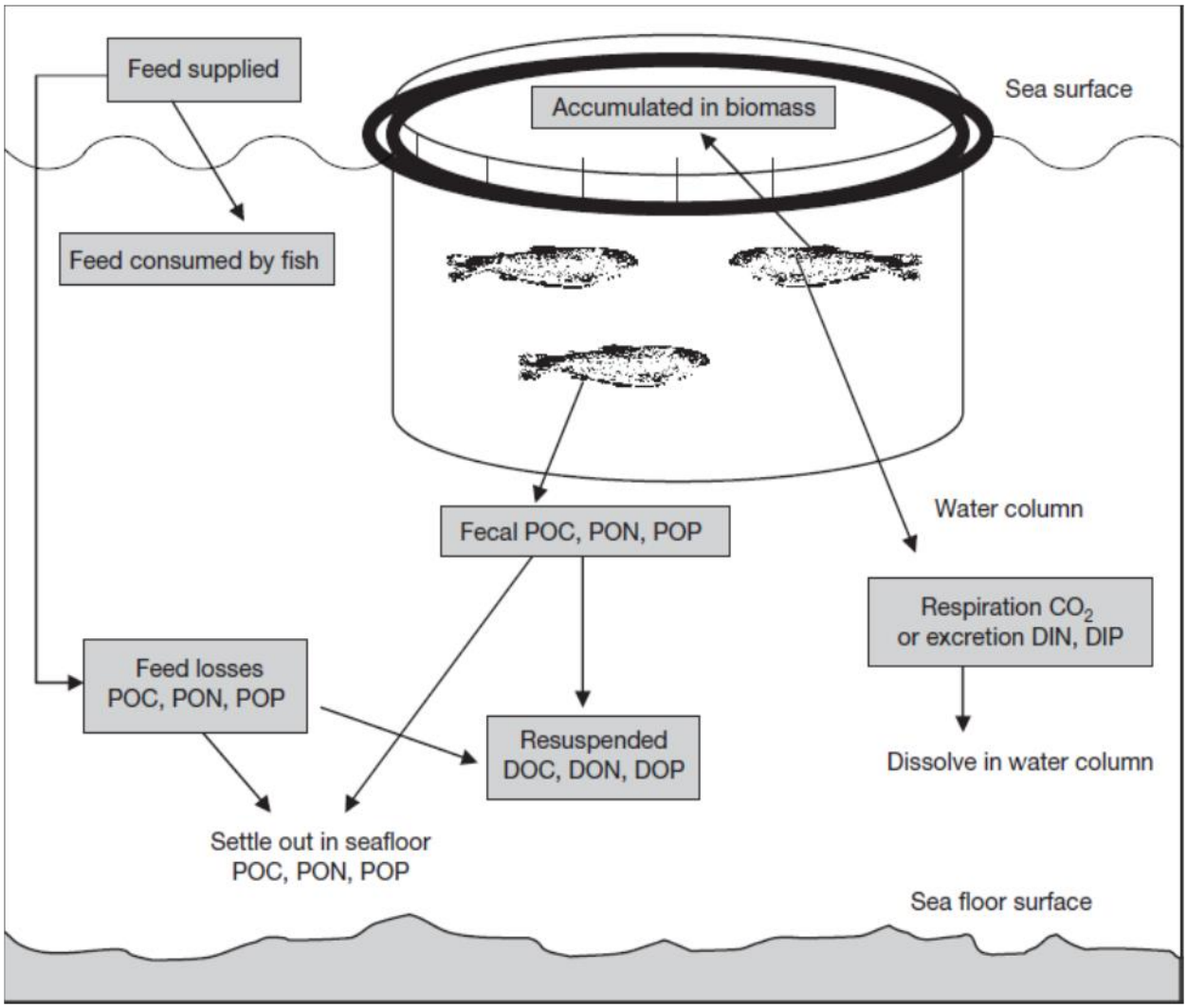

Figure 2-3: Nutrient waste cycle in aquaculture farm (Aquaculture, 2018)
POC: Particulate organic carbon; PON: Particulate organic nitrogen; POP: Particulate organic phosphorous; DOC: Dissolved organic carbon; DON: Dissolved organic nitrogen; DOP: Dissolved organic phosphorous; DIN: Dissolved inorganic nitrogen; DIP: Dissolved inorganic phosphorous

2.4 Reduce environmental impact

Generally, the stability of the pellets in sea water with immersion depends on the physicochemical parameters of ingredients used. Quick degradation of feed pellet can reduce by using attractant substance in fish feed formulation, oil spray on surface reduce the disaggregation of pellets, increasing hardness of pellet by reducing the size. Eventually this reduce nutrient leaching and increase the meat quality of fish by eating full feed pellets (Yandi and Kurtoğlu, 2016). But (Chen et al., 1999) shows that smaller pellets break easier than larger ones. Therefore, may be this hardness comes with the method of processing than the size. Therefore, need to use better technologies, formulations, and equipment while processing. When consider about the feed intake by fish, because of less digestibility of harder pellets, the feed intake is about 20% lower in harder pellets (Aas et al., 2011). Ideal hardness of pellets need to reduce environmental effect (Yandi and Kurtoğlu, 2016). Therefore, more studies needed to figure out this hardness of pellet and environmental damage loss.

Some extruded pellets have less density than water and not sink for long time. Due to the extrusion process the pellets contain air bubbles and pellets sink after filled these air bubbles with water. Therefore by changing the characteristics of pellets can reduce lots of feed losses in fish farms (Yandi and Kurtoğlu, 2016).

About 20% feces production reduction occur with introduction of high energy feed varieties with low pollution (Chen et al., 1999) Feces from healthy salmon fishes are stable due to mucous coverage around the feces and not degrade quickly (Merceron et al., 2002).

The dissolved nutrients can be reduced by increasing the rate assimilation process to reduce the severe environmental impact to the pelagic ecosystem occurring hypernutrification (Black, 2001, L Carroll et al., 2003). Current create by raft can take the suspended matter to the surface, giving less time to convert ammonia in to nitrate or nitrite (Merceron et al., 2002, Ervik, 1985). In cages fish feces, uneaten fish feed and oxygen depletion due to high number of fish is reduced by water changing (Merceron et al., 2002).

It is important to monitor ability of a body of water to cleanse the water bottom itself (assimilation capacity/ flushing capacity) to reduce the effect. For analyzing the monitoring process (L Carroll et al., 2003)mentioned four expensive methods used in Norway as;

- Visual diver survey
- Faunal analysis
- Sediment chemistry
- Sediment profile imagery (SPI)

With the organic matter accumulation faunal communication quantitatively increase in aquaculture system. Therefore, quantitative faunal analysis is one of the best method to determine the environmental impact occurs with fish wastes (L Carroll et al., 2003).

But these methods usages depend on the financial capacity of the fish farmers, availability of the resources and the area of the farm and so on.

However, using novel technologies to monitor the feeding system as camera controlling system the waste comes from feed is now is lesser than 5%, showing better image for the environmental and economic loss in salmon aquaculture lesser than fecal waste. Further environmental friendly ingredients using is an another option to reduce damage to the environment by feed (Wang et al., 2013, Yandi and Kurtoğlu, 2016). Novel test improvement as DORIS test, portable testers as Holmen pellet tester (see Figure 2-4) use to check the pellet degradation during transport. By using these test methods can avoid feed waste using degraded pellets.

(L Carroll et al., 2003) says analyzing lots of fish farm sites in Norway, the periodic management practices to recovery is one of the best solution for better environment than considering the depth or speed of the current. Because the high water exchange capacity is now available in Norway in fish farms, but the environmental impact is high due to increase of waste production rate (Wang et al., 2013).

Because of large nutrient portion in waste comes from feed, it is important to make action to correctly usage of fish feed with reduce feed conversion ratio (FCR) as now FCR is less than 1.2 lesser than 1993 (1.7) (Wang et al., 2013). By changing the formula in feed is now using to reduce waste as feces from 30% to 15% in fish industry (Piedecausa et al., 2009).

Figure 2-4: Pellet durability tester (TekPro, 2018)

The national criteria for characterize the effect of waters, water quality standards, standards for monitoring system is established in 1997 and 2000 in Norway to reduce the environmental impact in fish farms (L Carroll et al., 2003, Ervik et al., 1997, Maroni, 2000, Hansen et al., 2001).Annual inspection of sea cage fish farm is done by the Norwegian R&D institute called Akvaplan-niva (L Carroll et al., 2003).

The fish waste can be food source for another set of species in food chain (Cheshuk et al., 2003), as co- culture as macroalgae to reduce soluble inorganic nutrients , to maintain the water quality , culture blue mussels, sea cucumbers to use particle wastes (Cheshuk et al., 2003, Troell and Norberg, 1998, Whitmarsh et al., 2006). But Blue mussels can't compete with sea weed and phytoplankton due to lack of particulate matter in the beginning and waste generated by itself to the water body (Elberizon and Kelly, 1998). Bivalves in less nutrients area use more wastes (Handå et al., 2012, Wang et al., 2013). Sea cucumber co-culture with salmon can do better work by using uneaten feed and other unused particulate matter in the bottom (Uthicke, 1999, Michio et al., 2003, Paltzat et al., 2008).

This kind of aquaculture system which is dependent on each other species called IMTA (Integrated multi-trophic aquaculture) (Chopin et al., 2001, Abreu et al., 2011, Wang et al., 2013, Petrell and Alie, 1997, Marinho-Soriano et al., 2009).

When consider about the digestibility of phosphorus in fish feed with animal origin higher than in plant origin feed due to lack of phytase enzyme inside the fish body to digest plant phosphorous and release as particulate wastes which is good food source for blue mussels, sea cucumbers (Wang et al., 2013, Hua and Bureau, 2006).

Nitrogen loss about 60% of the feed basically as ammonia with urea, quickly removed by macrolage and phytoplankton (Wang et al., 2013). However, the overgrowth of macrolage and phytoplankton cause negative environmental impact as eutrophication. Therefore IMTA with good monitoring system with reducing the weed waste is a combined best solution. (Wang et al., 2013) says IMTA system is facilitated by both fish feed and higher portion of feces waste in salmon aquaculture system.

2.5 Settling rate of fish pellets and feces

Before determining the settling velocity of feces and feed in laboratory experimental setup normally the feed and fecal pellet keep at freezing temperature like -20 C. Then weight of the pellets takes after thawing them. Perpex tube (1.5 m length and 10 cm diameter) use to determine the settling velocity. During the experiment the tube keep in vertical position. After filling the tube with sea water, to keep the pellet below the water level forceps is used. Then the time for pellet to travel 1m distance of tube is measure using a timer. Eventually using the travelling distance and time the settling velocity of pellets are calculated. During the experiment the temperature of water is maintain a constant level (Chen et al., 1999, Law et al., 2014, Piedecausa et al., 2009, Pérez et al., 2014, Yandi and Kurtoğlu, 2016). Because according to the temperature the viscosity and density of the sea water vary.

When temperature increase about 10 °C the settling velocity increment is about 40%. When the water becomes warmer the density of water become lower and then the density of water in particle aggregate also become low. Therefore, the density of the flocs reduces in warm water.

When considering a particle in marine environment particle size, weight (Piedecausa et al., 2009) and density is the key factor for determining the sinking velocity of a particle (Bach et al., 2012). Further depend on shape and porosity of particle, temperature and viscosity of sea water (Chen et al., 1999).

After a non- extruded feed pellet soaking with water the dimension of the pellet is changed in higher percentage in small size pellets. After one minute soaking due to higher surface/ volume ratio smaller pellets achieve about 65% weight increment and 60% volume increment and respectively for larger feed pellets it is about 30% and 25 % (Piedecausa et al., 2009). (Vassallo et al., 2006) shows that after 10 minutes the weight increment of pellet is about 42% and the water absorption is depend on the feed composition of pellets. There is a relationship between pellet size and the hardness with the increment of size (Yandi and Kurtoğlu, 2016). By this the settling velocity of feed pellets reduces with high friction and high resistance to fall. (Piedecausa et al., 2009) shows during soaking changes in feed pellets (non-extruded pellet) and settling velocity of feed pellets have no relationship with the density of the pellets and temperature of water. But due to physical characteristic changes in feed pellets the settling velocity reduces with increasing immersion time by increasing diameter of the pellets and decreasing density of feed pellets. Generally feeds for salmon fish farms are extruded pellets. (Yandi and Kurtoğlu, 2016) says the water rate of absorption is increase with temperature increment of water and decrease with the increase of salinity of sea water.

Further (Chen et al., 1999) shows density of feces is good point to get an idea about feces settling velocity but not the length and diameter alone. The salinity of water, temperature, water current, and depth can affect for settling velocity of feces than fish type and size of feces. The feces pellet disaggregation become higher due to water waves created by fish swimming. But (Piedecausa et al., 2009) shows there is no relationship between settling velocity of feed pellet and temperature of water. Horizontal distance of particles to the benthic zone determine by the depth, current speed, size of the particles with settling velocity of particles.

Normally in cages in fish farm give same feed type (Piedecausa et al., 2009). Therefore, organic matter content and density is generally same in each fish farming site.

If settling velocity of a particle depends on the shape, dimension of pellet and density, viscosity of the medium, Stocks' Law can apply. Considering spherical shape particle, Stockes' law can apply to get terminal velocity of the particle if the Reynold number of sinking particle below 0.5. With temperature variation Stokes' low is with temperature dependent term as given in equation (2.1) (Bach et al., 2012);

$$f(T) = \frac{\rho_p - \rho_{sea\ water}}{\mu_{sea\ water}} \tag{2.1}$$

This temperature effect is applicable for other shapes particles as well. But (Chen et al., 1999) settling velocity changes with viscosity and temperature not goes with Stocke's low. According to (Vassallo et al., 2006) floating time before sinking also influence settling velocity of pellets. But (Piedecausa et al., 2009) it is not going with real farming environment with movement of fish and water.

Due to lower distance between the wall of the tube and particle the sinking velocity become low. Therefore, diameter of the tube is important to consider. It is better to keep the pellet on the middle of the water circle using forceps (Bach et al., 2012).

For some experiment to determine the fecal settling velocity the fecal material collect directly from rectal section of the gut of fish after killing one or two fish. This is more accurate when only need to determine fecal settling velocity without absorbing water, because the sample collects before mixing with water. After mixing with water the settling velocity decreases due to absorbing water and disintegration (Chen et al., 1999).

The sinking velocity of fish feed is depending on the size of the fish pellets and it is about 3± 15 cm/s (Chen et al., 1999, Merceron et al., 2002). And the sinking velocity of fish feces is about 2 ± 4 cm/s (Chen et al., 1999). According to the fish species the settling velocity of feces vary(Pérez et al., 2014, Piedecausa et al., 2009) shows that the settling velocity of feed pellets about 60 % higher than feces. Settling velocity of feces is lesser than feed pellets and spread over higher area than feed.

For small particles which are having Reynold's number less than one equation (2.2) is used to calculate setting velocity and mass of particles determined by equation (2.3) (Pérez et al., 2014, Yandi and Kurtoğlu, 2016).

$$V_s = \frac{1}{18} g \left(\frac{\rho_p - \rho_a}{\mu}\right) d^2 \tag{2.2}$$

$$m_p = \left(\frac{18 V_S \mu}{g d^2} + \rho_a\right) V_P \tag{2.3}$$

But this method has lots of limitation as spherical shape, low Reynold's number, laminar flow and so on. To get the relationship between the shape and settling velocity, different shapes as spheres, spheroids, ellipsoids must study.

As example Boekhout shape factor can use for ellipsoid shape particles as given in equation (2.4) where, n is a number between 0 and 1 while $D_s, D_{l,} D_i$ are short, intermediate and long axes of the ellipsoid respectively.

$$BSF = \frac{D_s}{D_l^n D_i^{1-n}} \tag{2.4}$$

This is accurate for the value 6 for "n". To check exactly the Boekhout shape factor for the relationship between shape of the particle and settling velocity more experiments needed. There are other shape factors as Le Roux shape factor (LRSF), Hofmann shape entropy (HSE), Corey shape factor (CSF). Further experiments needed to get an exact equation to explain accurately

the relationship between shape of the particle and settling velocity of the particles (Boekhout, 2012).

The settling velocity of feces and feed pellet are concluded in Table 2-5 (Chen et al., 1999, Findlay et al., 1995, Vassallo et al., 2006, Piedecausa et al., 2009).

Table 2-5: Settling velocity of feces and feed pellets

	Settling velocity of feces and feed pellets		
	Weight (g)/diameter (mm)	**Settling velocity (ms^{-1})**	**Reference**
Salmon Feces	0.04-0.09 g	0.053-0.066	(Chen et al., 1999)
	0.13-0.22 g	0.051-0.064	
Seabream &Seabass feces	0.02-0.74 g	0.022-0.075	(Piedecausa et al., 2009)
Seabream &Seabass feed	3mm	0.087 ± 0.0008	(Vassallo et al., 2006)
	5mm	0.144 ± 011	
Salmon Feed	3mm	0.055	(Findlay et al., 1995)
	10mm	0.155	

2.6 Effects of settling rate of the fish feed and fish feces

To get an idea about the environmental damage of aquaculture farms the information bout amount and composition of waste is not enough, information about settling velocity of waste particles, stability and leaching nutrient amount also important to know (Chen et al., 1999).

When feed particle or feces particle settle in marine environment leaching of organic compounds occur and fast initially. After a particle detached into small particle, the leaching rate increase with increasing surface area of the feed or feces particle. Generally, when considering same weight feed and feces particles leaching rate of ammonia in feces particle is three times higher than feed particle. Even though few studies were done to study the leaching during particle settling of particle in salmon fish farming, one or two studies were done for wild fish and Mediterranean fish sites (Piedecausa et al., 2009).

In fish farm, after feed pellets break in to particles, the particles tend to agglomerate together feces particles and other waste particles by flocculation process. Therefore, the slower small particles having lower sinking velocity become increase with increasing density. Density

increment basically occurs with dust, calcium carbonate, silica which come from fish pellets. This agglomerate particle calls as "flocs". The rate of agglomeration is increase with the number of large particles, stickiness of particle, slow turbulence and also with the help of zooplankton. These agglomerated particles not only include fish feed particles it also includes feces and other particles as well. The normal settling velocity for flocs is about $0.001 ms^{-1}$ and increase up to $0.1\ ms^{-1}$ with the water current (Law et al., 2014, Bach et al., 2012).

Sometimes contaminants transport to other fields about 2 km or to marine environment by these flocs. Filter feeding organisms as Mussles, scallops who lives in benthic level take this high organic matter as organic carbon from flocs. The time takes organic matter to sink to benthic level is higher with flocs than alone. If flocs contain hazard particles these benthic organisms become contaminate easily (Bach et al., 2012).

More sophisticated methods now available for determine sinking velocity of microscopic particles individually in a mixture of particles as video microscopy. In this method samples take into cuvette and using microscopic camera take photographs of particles during the sinking period (Bach et al., 2012).

2.7 Models regarding aquaculture waste and settling velocities

With the availability of resources, situation, severity of the environmental effect, availability of expertise, and also with the financial capability the monitoring system and combination of the monitoring system need to be used to get the optimum positive results (L Carroll et al., 2003). By model development using expert knowledge, using monitoring system and using available data, can predict the waste generation, oxygen depletion, environmental damage before starting the aquaculture farm or while doing the farm.

(Wang et al., 2012) have determined the ratio between carbon, nitrogen and phosphorous in waste in Salomon aquaculture system, by using a mass balancing model with the help of other literature as well. In this model carbon, nitrogen and phosphorous content of fish body, fish feces and fish feed have used. These model have been used and further developed for Integrated multi trophic aquaculture (IMTA) system using another set of coefficient value (L Carroll et al., 2003, Wang et al., 2013).

To get a model to study about environmental effect of aquaculture waste information about the settling velocity, agglomeration, mass and density of the feed and feces particles are important. These models regarding benthic waste input and distribution are important in future to predict about the environmental effect and it is cost effective (Law et al., 2014, Pérez et al., 2014, Bannister et al., 2016).

Settling velocity of feed and feces of sea bream and sea bass use in model MERAMOD (Pérez et al., 2014, Cromey et al., 2012). Models related to settling velocity of fish feces and pellets suggest that feed pellet spread ability is lesser than feces around the farming area (Piedecausa et al., 2009). Simulation of model for Atlantic Salmon fish farm and spread of waste into fjord system give results as more than $3/4^{th}$ of organic matter waste spread near to the farm and about 3% of organic waste spread to far areas from the farm (Bannister et al., 2016).

DEPOMOD model develop for prediction of accumulation of solid and community in sea bed in fish cage. Further to select a position for fish farming, to get an idea about the biomass content in sites this model is important MERAMOD model also develop to get the relationship between particles accumulation and benthic communication in Mediterranean aquaculture system (Cromey et al., 2012). The environmental effect of Atlantic cod farming in Shetland,

UK is model by (Cromey et al., 2009) as CODMOD. This is developed using DEPOMOD model (Richard Moccia, 2007).

When add new culture species to fish farm the effect of the environmental effect study by modeling with species specific parameters such as feed type (Vassallo et al., 2006). If not the model not give accurate results (Pérez et al., 2014).

Other than settling velocity, the depletion of oxygen by biodegradation of pellet using oxygen can describe by non-linear model as NL-MOD and linear model as L–MOD.

In NL-MOD describe oxygen usage depend on the organic matter present in benthic zone which can use by nonlinear Monod kinetics for growth of bacteria. Here organic matters divide into degradable and non-degradable organic matters. In this model nitrification and denitrification processes can added to get oxygen utilization changes with varying protein content according to the composition of feed.

In L-MOD says the decomposition of compound proportional with the available amount of each compound without separating degradable and nondegradable portion. Therefore, when large amount of organic matter available in system, it gives high oxygen utilization value than used value.

Degradation of dead microorganisms also should add to these two models for using these for longer time period (Klanjšček et al., 2012).

To study the Phosphorous cycling with eutrophication, simple empirical relationship and sophisticated dynamic models are used (Istvánovics, 2010).

2.8 BOD Test

In 1908 UK Royal commission on river pollution named Biological oxygen demand (BOD) as an indicator of organic pollution in rivers for 5 days period considering the time takes water to meet sea.

In this scenario aerobic biological organisms used dissolved oxygen and breaking down organic material present in the sample through respiratory process at specific temperature over a specific time period., called as carbonaceous demand (Roppola et al., 2007). Generally, this is expressed as milligrams of oxygen used per liter of given sample. Incubation temperature 20 °C and 5 days period used in general BOD test mostly in the past time (Jouanneau et al., 2014, Roppola et al., 2007, Nagel et al., 1992).

The reaction involve in complete degradation of organic matter is given in equation (2.5). This is used to get carbonaceous theoretical oxygen demand (CTOD)(Klanjšček et al., 2012).

$$\frac{1}{d}C_nH_aO_bN_c + \frac{1}{4}O_2 \rightarrow \frac{n-c}{d}CO_2 + \frac{c}{d}NH_4^+ + \frac{c}{d}HCO_3^- + \frac{a-5c}{2d}H_2O \qquad (2.5)$$

During aerobic biodegradation process organic matter biologically oxidize into biodegraded products, CO_2, and H_2O (Tan and Lim, 2005, Pagga, 1997, Reuschenbach et al., 2003).

During BOD test rather than organic material inorganic ions as sulfide and ferrous are oxidized using oxygen to oxidize reduced nitrogen. This is called nitrogenous demand. This can be calculated theoretically and called nitrogenous theoretical oxygen demand (NTOD) (Klanjšček et al., 2012). Overall reaction for NTOD is given by equation (2.6).

$$NH_4^+ + 2O_2 + 2HCO_3^- \rightarrow NO_3^- + 2CO_2 + 3H_2O \quad (2.6)$$

This should be avoided by adding inhibitors before starting the test without affecting for the accuracy of the test. (Roppola et al., 2007, Liu et al., 2000).

The total of NTOD and CTOD, name as theoretical oxygen demand (TOD) (Klanjšček et al., 2012).

BOD is important as a main indictor for waste water discharge and along with COD (chemical oxygen demand) to get an idea about the biodegradable portion of waste samples. Further the COD and BOD ratio determine the size of the treatment plant. BOD analysis results are mostly needed for standardizing purposes and controlling purpose of aerobic water treatment plant.

2.8.1 Methods used for BOD testing

Three methods as manual, semi-automated, and closed bottles test are available to measure BOD in the samples. In the manual method BOD measurement is done after incubation the aerated, sealed bottles with added microbial content at 20 °C for predetermined number of days and determines the dissolved oxygen at the end using iodometric method (Winkler's method), Skalar – Holland (Jouanneau et al., 2014).

These days there are more developed techniques to BOD measurements than conventional test as iodometric titration method (ISO 5813:1983, Water quality - Determination of dissolved oxygen - Iodometric method) which is not have online monitoring system and slow with sample preparation. But conventional test is not costly as novel methods and it is universally recognized method for BOD measurement and give comparable results (Roppola et al., 2007, Liu and Mattiasson, 2002). Other than iodometric method BOD measurements is done by oxygen sensor determination , electrochemical probe determination (ISO 5814:1990, Water quality - Determination of dissolved oxygen - Electrochemical probe method), UV absorption measurement, manometric respiration and so on. For analysis of oil sample using spectroscopy surfactant used as Triton X-100 (Kuokkanen et al., 2004).

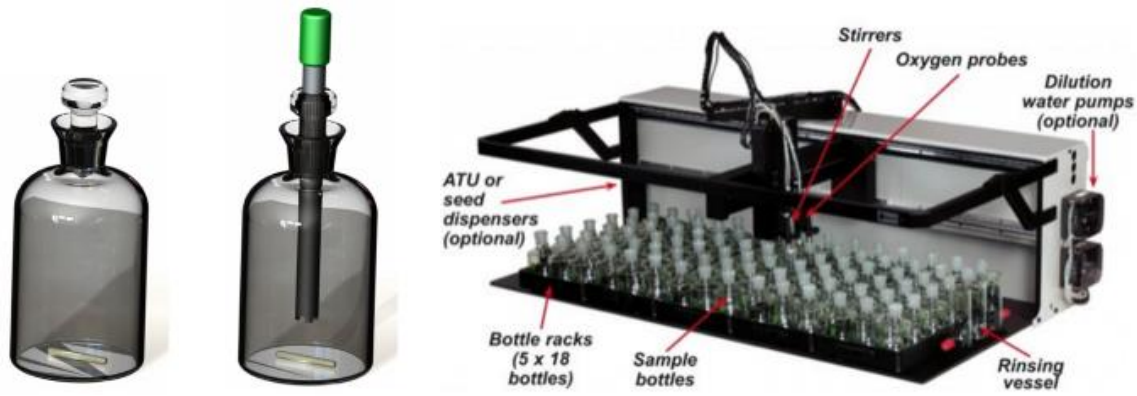

Figure 2-5: From left to Right: Manual, semi-automated and automated bottle test for BOD (Jouanneau et al., 2014)

In semi-automated and closed bottles methods, continuously measure the oxygen usage by microorganisms in the sample tested using an electrochemical probe and in addition in automated bottle test, bottles placed on the robotic analyzer, Skalar – Holland (Jouanneau et al., 2014) (see Figure 2-5).

Novel technologies for BOD testing come into the analytical world using biosensor /biofilm, bioluminescent bacteria using cellular activity, bioreactor, redox mediator and microbial fuel cells (which are used respiratory activity) for BOD measurement using less time. Most of them have online monitoring system, low maintenance and easiness. But more and more field applications, researches needed for make sure about the accuracy (Jouanneau et al., 2014, Liu and Mattiasson, 2002).

In biosensors method (biofilm and respirometer method) measurement takes using biochemical receptors. These receptors indirectly contact with transducer (Liu and Mattiasson, 2002)

As example due to less time consumption, inexpensiveness, easiness, online monitoring system, there is more focus on biofilm type usage for BOD testing.

In this kind of system immobilized microbial population or microbial film is used in the middle of a porous cellulose membrane and a gas permeable membrane. The microbes in the film bio oxidize the organic material. The quantification done by a physical transducer in relation to the presence of biodegradable material (Liu and Mattiasson, 2002).

The selection of the appropriate test for BOD measurement have to be done by considering errors, measurement frequency, according to the sample, money amount and application method as on line, in line and so on (Jouanneau et al., 2014).

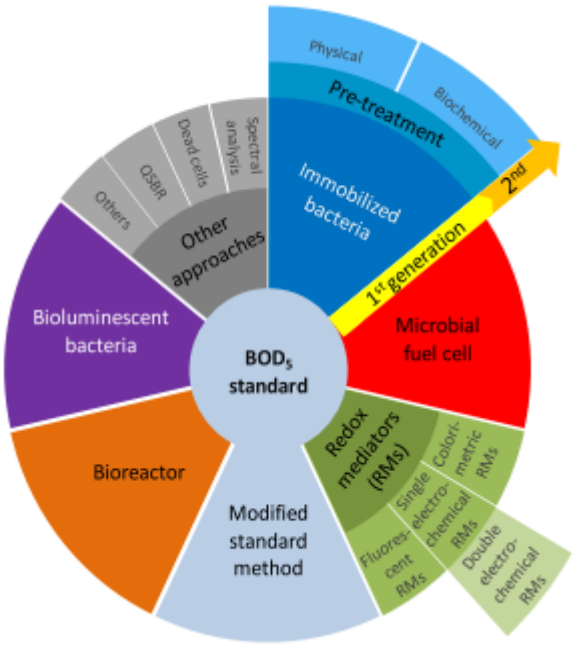

Figure 2-6: New methods to estimate BOD_5 (Jouanneau et al., 2014)

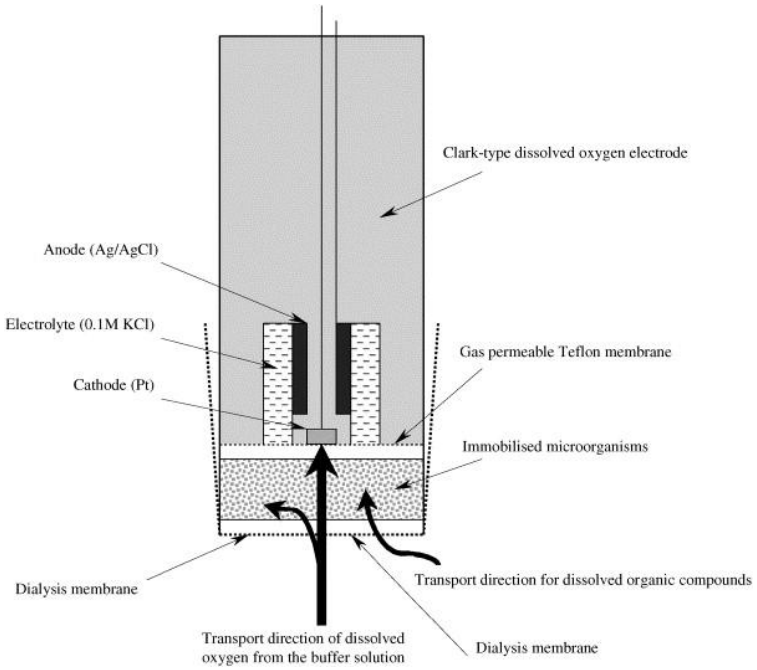

Figure 2-7: Diagram of a BOD biosensor (Liu and Mattiasson, 2002)

2.8.2 OxiTop Method

Caldwell and Langelierin 1948 developed the method named as manometric method for BOD measuring using the pressure reduction due to oxidation of organic substances using oxygen by microorganisms. In this experiment, first bottles are filled using the predetermined volume of sample and closed them. Then microorganisms can only use the oxygen inside the bottle and produced carbon dioxide by microorganisms absorbed into sodium hydroxide pellets which are inserted to touch with headspace of the bottle. During the experiment the pressure changes can be measured using a manometer and give as oxygen consumption value (Jouanneau et al., 2014).

A mercury barometer is used to measure the pressure difference and the conversion to oxygen is done by a graduated scale. OxiTop (WTW Weilheim, Germany) is one of the pressure sensor without using mercury but experiment time is more or less same (Roppola et al., 2007). Other than this other technically improvement reference methods are quick scan BOD analyzer, BOD track, Oxi Direct and CI-B 5 BOD analyzer are used as sensing pressure difference in the world-wide experiments. Further because of enough oxygen inside the bottles if samples contain high amount of carbon without diluting the sample BOD can measure by these sensor methods (Jouanneau et al., 2014).

2.8.2.1 Manometric respirometry test with OxiTop control system

BOD OxiTop method is accurate method to get an idea about aerobic degradation in chemicals (Kuokkanen et al., 2004). When consider about the general procedure the filled bottles with known amount of sample volume are sealed quickly with a rubber sleeve with carbon dioxide absorber as sodium hydroxide pellets. Further n-allylthiourea (ATU) is added as nitrification inhibitor as equation (2.7) (Roppola et al., 2007).

$$CO_2 + 2NaOH(s) \rightarrow Na_2CO_3(s) + H_2O(l) \quad (2.7)$$

Then measuring heads are caped to the bottles and sample bottles keep in the incubation cabinet normally at 20 °C + 2 °C for about two hours before start for stabilizing purpose. Magnetic stirrer is inserted into each bottle before closed and samples keep on the inductive stirring system inside the incubation cabinet. If it is short term test as BOD7 after 7-day period computer program used to get the results as Excel Sigma Plot and TBL curve (Roppola et al., 2006).The user can program the measurement time duration. During every test, a blank test is also carried out (Roppola et al., 2007). Here the measurement of pressure difference at constant incubation temperature is very accurately done by this method. The pressure reduction to measure BOD value occurs with usage of oxygen for oxidize the organic matter and reduction of produced carbon dioxide by absorbing to sodium hydroxide (Binner et al., 2012).

The BOD is expressed as mg/L using modified ideal gas low as shown in equation (2.8) (Kuokkanen et al., 2004, Roppola et al., 2006, Roppola et al., 2007).

$$BOD\ (mg/L) = \frac{M(O_2)}{RT_m}\left[\frac{V_{tot} - V_l}{V_l} + \frac{\propto T_m}{T_0}\right]\Delta p(O_2) \quad (2.8)$$

The volume of the sample in each bottle is decided according to the measuring range of the sample.

As example for 0–80 mg/L sample must use 365 mL water. Further nitrification inhibitors and additional microbial source must be done before fill the bottle and if necessary addition of mineral nutrient solutions also done (SFS 1889-1 standard, dilution factor 2). Nutrient solution prepares using chemical compound as KH_2PO_4, K_2HPO_4, $Na_2HPO_4 \cdot 2H_2O$, NH_4Cl, $CaCl_2$, $MgSO_4 \cdot 7H_2O$, and $FeCl_3 \cdot 6H_2O$ (OECD standardization organization). But finally balance the weight must be correct to have a constant volume.

Nitrification process first bacteria oxidize ammonium to nitrate and nitrate. Generally waste water sample contain very low amount of nitrogen or free ammonia and by this oxidation < 5 % contribute to total BOD (Roppola et al., 2006).

After the incubation period remaining oxygen is measured using iodometric method or electrochemical probe method (Roppola et al., 2006). The little variation of the above methods can be seen according to the analyzing sample. As example for testing BOD in natural ground water no nutrients, inoculums or nitrification inhibitors are added, called simulation test (OECD standardization organization) (Kuokkanen et al., 2004).

2.8.2.2 Nitrification in BOD:

During hydrolysis of protein ammonia produced. The ammonia is converted to nitrate in aerobic condition. This is called nitrification (see equation (2.9) and (2.10)).

$$2NH_3 + 3O_2 \xrightarrow{Nitosomonas} 2\,NO_2^- + 2H^+ + 2H_2O \quad (2.9)$$

$$2NO_2^- + O_2 \xrightarrow{Nirobacter} 2\,NO_3^- \quad (2.10)$$

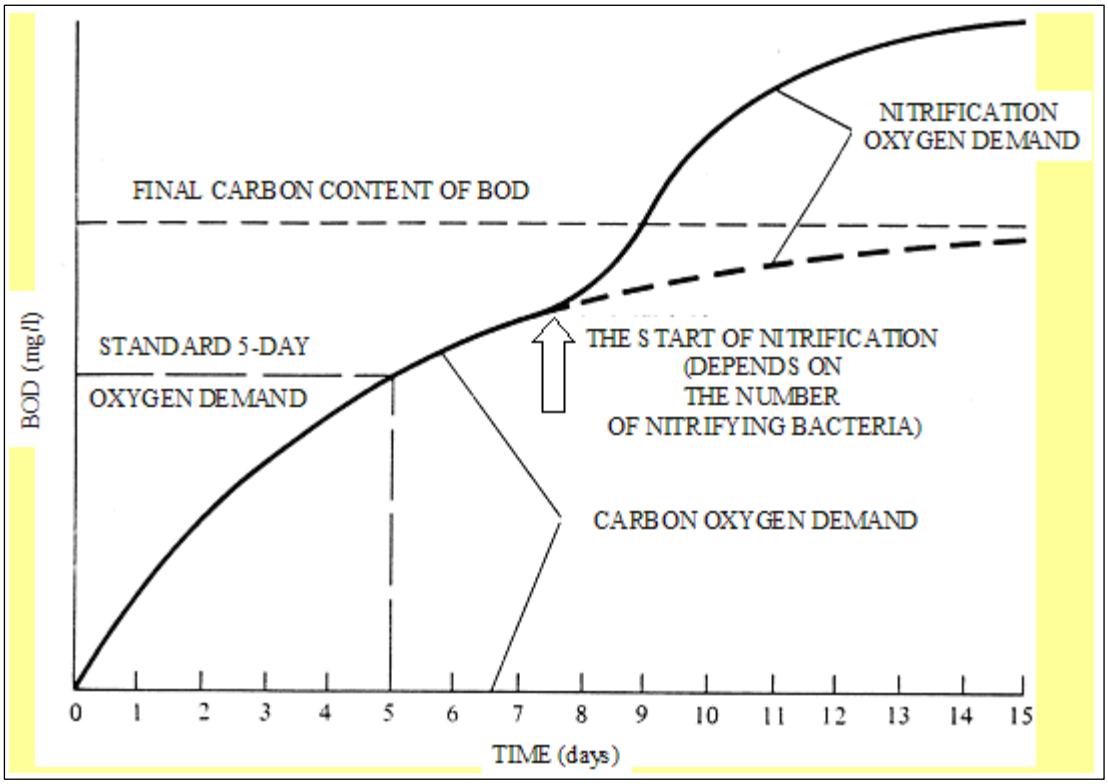

Figure 2-8: Nitrification in BOD (Csaba and Csaba, 2011)

Oxygen required for the oxidation of ammonia is named as nitrogenous BOD (see Figure 2-8). The growth rate of nitrifying bacteria this scenario occurs in day 6 to day 10 normally. Therefore the 5 days of incubation done for the BOD test to get rid from this effect in 5-day method (Themes river require 5 days to meet sea from its origin) and get carbonaceous oxygen demand. n-allylthiourea (ATU) is added as nitrification inhibitor in high duration tests (Roppola et al., 2007).

2.8.2.3 Degree of biodegradation

Using the OxiTop BOD value in milligram per liter can be convert in to milligram per milligram using following equation (2.11).

$$BOD\ \left[\frac{mg}{mg}\right] = (BOD\ [mg/L]\ x\ liquid\ phase\ volume\ [LD]/\ sample\ mass\ [mg] \qquad (2.11)$$

Then carbon content of the sample is measured as using PerkinElmer2400 series II CHNS/O analyzer. Here combustion of the sample is done, and carbon, hydrogen and nitrogen measured as gas CO_2, H_2O and N_2 respectively. There are other time consuming, hazardous, inaccurate methods such as Potassium dichromate method. Theoretical oxygen demand (ThOD) of sample is calculated by using equations (2.12), (2.13), (2.14), (2.15) where the degree of biodegradation is given by equation (2.16).

$$BOD\ [(mg/mg)] = [BOD[md/L].\ liquid\ phase\ volume\ [LD] / sample\ mass\ [mg]\ ] \quad (2.12)$$

$$m(C) = Carbon\ content\ [\%]\ x\ sample\ mass\ [mg]/100 \quad (2.13)$$

$$m(O_2) = m(C)\ x\ [M(O_2)/M(C)] \quad (2.14)$$

$$ThOD = (O_2)/sample\ mass\ [mg] \quad (2.15)$$

$$Degree\ of\ biodegradation\ [\%]\ =\ BOD/ThOD\ x\ 100\% \quad (2.16)$$

2.8.2.4 Benefits of OxiTop method by compare with other methods

When compare the results in some sludge sample with chemical method results, results from OxiTop is usually higher due to absorption of methanol, mercaptan and small organic acids in to sodium hydroxide and make extra reduction of pressure. In this kind of case little deviation can be occurred with other BOD tests (Roppola et al., 2006). Generally the total concentration effect from chemical degradation, evaporation and foaming and also anaerobic biodegradation can be prevented in the OxiTop method than other methods (Kuokkanen et al., 2004).

Therefore the precision is higher in OxiTop method due to accurate measurement of pressure with temperature control and homogeneous sample (Kuokkanen et al., 2004) than conventional method as using IR which is time consuming, use hazardous chemicals as CCl_4 for extraction using many samples. Biodegradability of oil in water sample (Kuokkanen et al., 2004)also can check by OxiTop method rather than using extraction series and the conditions are changed accordingly but method is not change with biology and composition of the sample. For the test only one sample is enough, easy to take samples and monitoring is done by the automatic system. Generally OxiTop method is easy, less effort need and can arranged quickly (Pisal, 2009).

OxiTop method shows the oxygen usage for degradation of organic compounds and can be compared with the oxygen consumption theoretically. But when compare with IR method even though it says about total hydrocarbon content, nothing says about reduction of hydrocarbon. As OxiTop method, IR method unable to separate between aerobic and anaerobic biodegradation, nitrification, and evaporation of volatile compounds. So the methods like IR methods have many limitations than OxiTop method (Kuokkanen et al., 2004).

Sometimes fast biodegradation within some samples as oil samples can slow and produce harmful products and harm to the microbes and make incorrect measurements, but it is need more researches to prove it because sample containing plant based bio oil biodegrade quickly without causing problems (Kuokkanen et al., 2004, Roppola et al., 2006).

2.8.3 Micro-Oxymax method

Micro-Oxymax respirometer (Columbus Instruments International Corporation, 1994) is a closed loop respirometer (see Figure 2-5). In the closed loop (sample chamber and gas analyzer) the head space gas in chamber is isolated, mix and pass through gas sensors and return it back (flow rate of 0.5 L/m) to chamber periodically.

It is possible to measure production or consumption of many types of gases in percentages even at very low level of concentration. These percentage values are used to calculate rates and cumulative gas production or consumption (Liebeg and Cutright, 1999). Microbial activity using O_2 and producing CO_2 measured in this instrument.

Micro-Oxymax used pressure sensors to get the volume of gases comparing with pressure reading of known volume in reference chamber. Here automatically used Boyle's law ($P_1V_1 = P_2V_2$).

At the beginning volume of the headspace and O_2 concentration are measured. When the oxygen concentration of head space goes below 10 % chamber is refreshed using ambient air (flow rate 4 L/m). Using 4hour sampling interval (defined by user) O_2 uptake rates (mg/h/ml) are measured using paramagnetic O_2 sensors. The maximum sensitivity is 0.2 μL/hour. This system has Ultra IBM compatible computer to collect and record data.

The condensing air dryer which is situated between sample and standard gas dryers, returned evaporated water back to sample chamber to maintain the moisture level (Liang et al., 2003). A pump is used in the system to pump air in and out.

2.8.4 Limitations of BOD test

For the BOD test high concentration of active bacteria need to do the process. According to the sample need to be tested pretreatments are needed. As example if the sample contain toxic compounds. Nitrification must reduce using nitrification inhibitors as pretreatment. By the BOD test biodegradability of inorganic materials can't be measured. Only organic biodegradability measured. During the test after organic substances used the test doesn't have valid stoichiometry. The test is time consuming (Tchobanoglous et al., 2002).

2.9 COD

Chemical oxygen demand (COD) can be used to have an approximate idea about theoretical oxygen demand (ThOD) which means the amount of oxygen use to oxidation of organic compounds into inorganic compounds totally. Generally, the organic compound oxidation is occurred 90-100 %. But if the sample contains compounds as in industrial waste water which are difficult to oxidize the value is not represent ThOD. During the reaction oxidization of organic compounds occurred rapidly excluding compounds with pyridine nucleus, quaternary nitrogen and so on. Some hydrocarbons having high volatility evaporate from the reaction place. Further bromide, iodide, nitrite, some metal, and sulfur compounds like inorganic compounds oxidized. Some are act as oxidizing agent in the reaction process and interference can occur with chloride content.

So, it is important to know the sample composition before prediction about ThOD using COD values. COD determined by dichromate method (Roppola et al., 2006). The organic matters oxidized chemically in a strong oxidant, dichromate acid solution.

Oxidation equation for reduces state of organic nitrogen are given in equation (2.17) (Tchobanoglous et al., 2002).

$$C_nH_aO_bN_c \ + d\ Cr_2O_7^{2-} + \ (8d + c)H^+ \rightarrow nCO_2 + \frac{a + 8d - 3c}{2} H_2O + cNH_4^+ + 2dCr^{3+} \quad (2.17)$$

$$where\ d = \frac{2n}{3} + \frac{a}{6} - \frac{b}{3} - \frac{c}{2} \quad (2.18)$$

ISO mentioned a method for COD determination of water which is for sample with COD 30 - 700 mg/L range and chloride more than 1000mg/L. If the COD is more than 700 mg/L the sample should be diluted to 300 -600 mg/L range to get accurate results.

According to ISO 6060:1989, COD in water is when the water sample reacts under defined condition, the amount of oxygen equivalent with the amount of dichromate used by the oxidation of dissolved and suspended matter (ISO 6060:1989, Water quality – Determination of chemical oxygen demand).

Filtration process is used to fractionate the COD of sample as soluble COD. It is basically depend on the pore size of the filter (Tchobanoglous et al., 2002).

Colorimetric and titrimetric analysis methods are used to measure COD in a sample. Colorimetric method is easier and faster method. Commercially available COD vials are used for the measurements. Chemometric assisted spectrophotometry method available now to measure the COD. Using relationship between multi wavelength signals of sample and corresponding concentration model is developed. Then the machine automatically catches the most sensitive wavelength and measures the COD in unknown sample (Alam, 2015).

In spectrophotometry cell test method inside the vials sample is oxidized with hot sulphuric solution of potassium dichromate with silver sulfate as catalyst to oxidize low molecular weight fatty acids. Then low Cr_2O_7or green Cr^{+3} is measured spectrophotometrically.

2.9.1 Difference between COD and BOD values

COD measurement is less time consuming than BOD measurement. Generally, these two values have a gap. Organic substances as lignin oxidized chemically but difficult to oxidize biologically. In the test some inorganic substances are oxidized by dichromate (Roppola et al., 2006) by these oxidized inorganic substances the organic content of the sample is increased and also due to the reaction of inorganic substances with dichromate increase the COD value. During BOD test some organic matters toxic to the microorganisms (Tchobanoglous et al., 2002).

2.10 Total, Fixed and Volatile solids

The material residue retains in the container after evaporation of sample inside an oven at defined temperature called as “Total solids” (see equation (2.19)). The combination of “Total suspended solids” and “Total dissolved solids” is named “Total solid”. When a sample is filtered through a filter the portion of solid retained by filter (2 µm or less) is called “Total suspended solids” (see equation (2.20)) and the portion of solid pass through the filter is called “Total dissolved solids” (Howard, 1933). The volatized portion after ignition of total suspended solid is called as “Volatile suspended solids”.

Total solid determination temperature is 105 °C for 12 hours in an oven. The heat dishes and watch glasses used in this procedure must follow the heating at 105 °C for 1 hour and cooling using a desiccator before used.

Weight of liquid samples should be weighed quickly as possible to reduce the evaporation losses. Watch glasses are used to cover the dishes without expose the sample to outside.

$$\%\, Total\ solid = \frac{W_{total,1} - W_{dish}}{W_{sample,1} - W_{dish}} * 100 \tag{2.19}$$

$$\%\, Total\ suspended\ solid = \frac{W_{total.2} - W_{FP}}{W_{sample,2} - W_{FP}} * 100 \tag{2.20}$$

The filtering procedure affected by the type of filter holder, porosity, size of the pores, thickness of filter and the physical nature, size of the particles, amount retained on the filter.

The residue of total, suspended or dissolved solids after heat to dry at specific temperature for specific time is called "Fixed solid" (see equation (2.21) and (2.22)).

$$\%\, Total\ fixed\ solid = \frac{W_{volatile,1} - W_{dish}}{W_{sample,1} - W_{dish}} * 100 \tag{2.21}$$

$$\%\, Fixed\ suspended\ solids = \frac{W_{volatile,2} - W_{FP}}{W_{sample,2} - W_{FP}} * 100 \tag{2.22}$$

"Volatile solid" is the weight loss occurred during ignition period. This fixed and volatile solid is not limited to organic matter; it includes decomposition or volatilization of some mineral salts as well (see equation (2.23) and (2.24)).

$$\%\, Volatile\ solid = Total\ solid - Total\ fixed\ solid \tag{2.23}$$

$$\%\ \text{Volatile suspended solid} = Total\ suspened\ solid - Fixed\ suspended\ solid \tag{2.24}$$

Generally, ignition temperature for determination of volatile solids is 550 °C for 2 hours in a muffle furnace. The evaporating dishes and watch glasses used for the ignition procedure must heat at 550 °C for 1hour in a muffle furnace and cooled in a desiccator before used (Agency, 2001).

2.11 Identification of knowledge gap and how that knowledge gap is filled

According to the literature study, the studies not show the combination effect for the environment from aerobic degradation of fish pellet and feces, settling rate of fish waste as feces and uneaten fish pellet, dissolution rate and so on. Many studies only focus for environmental effect from only one of the above-mentioned areas. It is difficult to get a correct idea about the overall negative environmental effect by combining these findings because these

researches are conducted in different countries with different water systems and using differently formulated pellets. And, there is not any good available model which cover everything regarding pellets and feces to identify the negative environmental effect.

In this study, chemical analysis of pellet and feces is conducted to get an idea on how much of ammonia, phosphate, total phosphorous, total nitrogen come to the fjord water with fish waste, try to identify the rate factor of aerobic degradation of uneaten fish pellets and feces, remaining inorganic and organic matter content available for negative environmental effect as eutrophication after almost complete aerobic degradation by bacteria in fjord water, amount organic compounds available for anaerobic degradation if only half aerobic biodegradation occur in benthic environment of fjord system, the settling rate of the feed pellet considering one meter depth in fjord water and how fat pellets come to benthic level for aerobic degradation by benthic bacteria, how physical disaggregation of pellet occur and effect for the settling rate of pellet, how this disaggregation increase the waste generation in fjord system by increasing amount of uneaten pellet and how disaggregation make easy for aerobic degradation by bacteria by increasing the surface area and so on. Therefore, in this study all these things combine and explain the bad environmental effect from fish feed and pellets. By using all these results together can create a good model for fjord water aquaculture system to identify the negative environmental effect than separate model for each aspect as describe in 2.7.

Further it can be described the reason of main and secondary objectives in this study thoroughly as follows;

2.11.1 Why determine the kinetics and degree of aerobic degradation in marine fjord water of typical fish feed pellets and farmed salmon feces?

It is important to conduct the kinetics and degree of aerobic degradation to get an idea about how much of organic and inorganic compound remain for negative environmental effect as eutrophication, saprobiation processes in fjord water. By determining the rate constant of degradation, an idea about the completion rate of aerobic degradation by microorganisms in fjord system with the availability of oxygen can be obtained. Also, the amount of organic matters that can go for anaerobic degradation in benthic environment due to not occurring complete aerobic degradation and what kind of worst scenario happen with anaerobic degradation than aerobic degradation can be investigated. It is also important to get a rate constant for aerobically degrade fish pellet and fish waste to develop a model, and to identify the negative environmental effect from uneaten fish feed and waste.

2.11.2 Why chemical analysis of fish feed and feces before and after aerobic degradation is conducted?

This is to get an idea about the amount of inorganic and organic matter come to the fjord water with fish feed and feces and availability of inorganic and organic matter after almost complete aerobic degradation by bacteria in fjord water. This available inorganic matter as ammonia. Phosphate, TP, TN go for process like eutrophication.

2.11.3 Why determine the settling velocity of fish pellets?

By settling velocity of fish feed can get an idea about how fast it can settle to the sea bottom and go to other fields to create environmental damages or outlet blockages in cage cultures. How disaggregation of pellet occurs during settling feed pellet to sea bottom. This

disaggregated particle easily biodegraded by bacteria than full pellet. And with soaking how physical properties like diameter changes occur in fish pellets and increase the surface area of pellets for aerobic degradation process. Further can know how fast it come to sea bottom and settle for the aerobic degradation by benthic bacteria after putting into fjord water.

2.11.4 Why determine the physical characteristics of pellets?

By knowing physical characteristic as diameter, weight can get an idea about the surface area availability in each different pellet sizes for aerobic degradation, to identify organic matter content variation in each pellet type for aerobic degradation with weight differences. To clarify the variation in setting velocity of each pellets due to shape, weight and also to describe soaking rates. And, how soaking rate of each pellet due to surface area to absorption fjord water delay or increase the settling of pellets.

2.11.5 Why determine the dissolution rate of the pellets?

To know how physical disaggregation, occur in each type of pellet and how it increases the aerobic degradation rate. To identify how each pellet disaggregation rate, give stimulation for the aerobic degradation process by breaking pellets into particles rather than organic content variation in each pellet with size and weight difference.

3 Material and methods

Under this chapter explain the site description from where the fish feed and feces come, the method use for test BOD and other chemical analysis, the way of do the settling velocity and degradation test. All together each step follows to do this study include in this section. Refer Appendix B for the images related to the material and experimental methods/equipment.

3.1 Study site

The experiment was carried out at University of Stavanger (UiS). The fish pellets samples were collected from Cargill Aqua Nutrition in Bergen, Norway which is a growing fish feed production company. Four different sizes of fish pellets were used for the experiments named as HP 1000 54 500 (P 1000), Ewos 500 S1 50 A (P 500), Adapt Marine 80 (P 80) and Ewos 200 S1 (P 200).

Fish sludge samples containing fish feces were collected from the salmon tanks of "FisterSmolt AS", which is located in fjords east of Stavanger. FisterSmolt does has a considerable amount of yearly fish production. One of major issue for them is the having a higher amount of sludge with fish feces in the outlet water. If this sludge directly released to the natural fiord water, it creates a major environmental impact to the ecological system as concentrated the nutrient content in water which leads to increase eutrophication (Merceron et al., 2002, Wang et al., 2013, Aure and Stigebrandt, 1990, Gowen, 1994, L Carroll et al., 2003, Stigebrandt et al., 2004, Kutti et al., 2007).

3.2 Sampling Procedure

Approximately 500 g of each fish pellets sample was transported to the laboratory of department of Chemistry and Life Sciences, Faculty of Science and Technology, University of Stavanger (UIS) and kept in dark place at -20 °C until the starting of the analysis procedure.

Fish sludge sample containing fish feces was kept in cold room (2 °C) in the laboratory of department of Chemistry and Life Sciences, Faculty of Science and Technology at UiS until analysis.

3.3 Fish feed solution

Pellets from freeze Extra HP 1000 54 500 (P 1000) sample were taken and crushed using Mortar and Pestle. 4.96 g of crushed sample was dissolved in 500 ml distilled water. The solution was mixed vigorously until all the particles fully dissolved. This solution was used for chemical analysis of fish pellets as representative sample for all type of fish pellets. It was seemed like the solution contain high amount of fat due to floating of oily layer on the top of the solution (see Figure 3-1).

3.4 Fish waste (sludge) sample preparation for analysis

The feces sample which is in a plastic container was opened and mixed using an electrical agitator (see Figure 3-2) to get a homogenized sample for analyzing.

Figure 3-2: Fish sludge sample

Figure 3-1: Dissolved fish feed solution

3.5 Chemical analysis of fish pellets and fish sludge

- pH of the solutions was measured by using a pH meter (pH/mV/°C meter, bench, pH 1000 L, pHenomenal®) before other experiments were begun.
- Total solids, Total fixed solids, Total volatile solids, Total suspended solids, Volatile suspended solids of prepared fish sludge sample and fish pellet sample were measured according to the standard methods.
- For Total solid measurement, known weight of each sample was measured in to the dishes. The initial dishes weights were measured before putting the sample. Dishes and watch glasses are oven dried for 1 hour and cooled in desiccators before used. Weighing is done by using electrical balance. Before and after weighing the dishes with samples were transferred covering with watch glasses to eliminate the evaporation. The samples were oven dried overnight and weighing was done by after cooling the dishes with dried samples in desiccators. Parallels were carried out to get the average to reduce the error.
- After weighing recording of overnight oven dried samples were kept in the muffle Furness at 550 °C and again samples were cooled in desiccators and measured the weights of the dish with samples. Finally, total solids and total volatile solids were calculated using equations (2.19) and (2.23).
- For fish pellet solution 1:100, 1:10, 1:5 and 1:2 dilution series were made by using volumetric flasks and distilled water. TCOD (Total chemical oxygen demand) and SCOD (Soluble chemical oxygen demand) were measured using standard COD cell test method. 100- 1500 mg/L range, Mercury free (Hg free) test cells were used for the test. 2 ml of sample was taken into a pipette and allowed to run from the pipette down the inside the reaction cell onto the reagent. The content was closed and vigorously mixed. After cells were kept in thermos reactor at 148 °C for 120 minutes, and after cooling to room

temperature (after 30 min) the measurements (absorbance corresponding to mg/L COD) were read by using the pharo 30 spectro-quant (Darmstadt, Germany).

- NH_4^+, N, NO_3, Total nitrogen (TN), PO_4, Total phosphorous (TP) were measured in filtrate and the solution using the standard cell test method.

3.6 Sample preparation for BOD tests

Biological oxygen demand (BOD) test was done by using OxiTop method and Micro-Oxymax method.

3.6.1 Method selection

Fish pellet composition was taken from the supplier. According to the composition, specific theoretical oxygen demand, and TVS, theoretical oxygen demand (ThOD) was calculated per one gram of pellet. Pellets (P 1000, P 500, P 200, and P 80) were weighed and calculated the ThOD for each size. Relevant method for BOD analysis was selected according to the ThOD (see

Table 4-2). The BOD of the pellets has higher ThOD were measured by using Micro-Oxymax and lower ThOD were measured by using OxiTop method.

3.6.2 Tests by OxiTop

Table 3-1: Arrangement of the bottles for OxiTop BOD test 1

Chamber number	Pellet/ Sample	Weight (g)
1	P 80 pellet 1	0.5389 + NaN_3
2	P 80 pellet 2	Glucose
3	P 80 pellet 3	0.0569
4	P 80 pellet 4	0.0952
5	P 200 pellet 1	-
6	P 200 pellet 2	Glucose
7	P 200 pellet 3	0.0552
8	P 200 pellet 4	0.0984
9	Positive control 1	0.0939
10	Positive control 2	0.0952
11	Negative control 1	0.0530 + NaN_3
12	Negative control 2	0.05389 + NaN_3
13	Blank 1	-
14	Blank 2	-
(+) ve control: 100 mg Glucose		
(-) ve control: P 80 pellet + 1 g NaN_3		
Blank: 400 mL sea water		

In experiment one, average weight of P 1000, P 500, P 200, and P 80 were taken using electrical balance.

BOD of P 200 and P 80 were done by OxiTop (WTW), (for principle see: sub chapter 2.8.2.1). According to the mass balance 400 ml sea water in one-liter bottles were selected for four parallels of two pellets Two positive controls, two negative controls and two blanks were also used. 1 ml of A, B, C, D nutrient samples were added for each bottle. For four bottles one pellet of P 200 and another four bottles one pellet of P 80 were added separately. 100 mg of glucose was included in positive control and a P 80 pellet and 1 g of NaN_3 were included in negative control bottles.

In second experiment, P 200 pellets, P 200 grinded pellets and P 200 grinded autoclaved pellet solution were used in 400 mL sea water as Table 3-2. The weight of the P 200 and grinded pellets in 1-4, 2-5, and 3-6 chambers were taken as equal. 10 pellets were added to 100 ml sea water (equation (4.26)) and autoclaved at 121 °C for 30 min under 15 psi pressure. From the autoclaved solution 10 ml were added to three chambers. One positive control and one blank were prepared using the same way as 1st experiment. One negative control was prepared using grinded P 200 pellet, 1 g of NaN_3 and 400ml sea water.

Sodium hydroxide granules inserted and placed in contact with headspace to absorb produced CO_2 in each bottle. Table 3-1 shows the arrangement of the test bottles. A magnetic stirring bar was inserted into each bottle. The bottles were kept on the stirring platforms inside an incubation cabinet (10 ± 1 °C) after closing each bottle with measuring heads. First the test was set for 14 days, then 1st experiment was continued another 7 days. Using controller, the amount of oxygen utilization values was taken by connecting with the heads of each bottles during and after the experiment. Finally, all the data were collected to an excel sheet plugging controller to a computer.

Table 3-2: Arrangement of the bottles for OxiTop BOD test 2

Chamber number	**Pellet/ Sample**	**Amount**
1	P 200 pellet 1	0.0971 g
2	P 200 pellet 2	0.0966 g
3	P 200 pellet 3	0.0960 g
4	P 200 grinded pellet 1	0.0971 g
5	P 200 grinded pellet 2	0.0966 g
6	P 200 grinded pellet 3	0.0960 g
7	P 200 grinded autoclaved pellet 1	10 ml
8	P 200 grinded autoclaved pellet 2	10 ml
9	P 200 grinded autoclaved pellet 3	10 ml
10	Negative control 1	0.0966 g + NaN_3
11	Positive control 1	Glucose
12	Blank 1	
(+) ve control: 100 mg Glucose		
(-) ve control: P 200 pellet + 1 g NaN_3		
Blank: 400 ml sea water		

3.6.3 Tests by Micro-Oxymax

BOD of P 500 and P 1000 were measured by a commercially available computer-controlled respirometer called Micro-Oxymax, Columbus Instrument Inc. (for principle see sub chapter 2.8.3). Here in 1st experiment, four parallels of two pellets with two positive controls, two negative controls and two blanks were prepared using 1 L bottles with 700 mL sea water and 1 mL of A, B, D nutrients. All together fourteen closed chambers were use. For four bottles separately one pellet of P 1000 was added after weighing them. For another four bottles one pellet of P 500 was added separately after weighing them using electrical balance. Four runs were made for each pellet type for reproducibility and reliability purpose. Positive control was included 400 mg glucose and negative control was included a P 500 pellet and 2 g of NaN_3 (see Table 3-3).

Table 3-3: Arrangement of the bottles for Micro-Oxymax BOD test 1

Chamber number	Pellet/ Sample	Weight (g)
1	P 1000,1	0.6139
2	P 1000,2	0.5170
3	P 1000,3	0.5850
4	P 1000,4	0.6492
5	P 500, 1	0.2551
6	P 500, 2	0.3105
7	P 500, 3	0.3136
8	P 500, 4	0.3016
9	Blank 1	-
10	Blank 2	-
11	(+) ve control 1	Glucose
12	(+) ve control 2	Glucose
13	(-) ve control 1	0.3033 + NaN_3
14	(-) ve control 2	0.2753 + NaN_3
(+) ve control: 400 mg Glucose		
(-) ve control: P 500 pellet + 2 g NaN_3		
Blank: 700 mL sea water		

For second experiment, 28 g of fish waste (Equation (4.20)) was used in three chambers with 700 ml sea water. For other chambers three P 1000 pellets, three grinded P 1000 pellets, three autoclaved grinded pellets were used with 700 ml sea water. The weight of 4-7, 5-8 and 6-9 were taken as similar Table 3-4. Further two negative controls, two positive controls and two blanks were prepared (Table 3-4). One negative controls were prepared using P 1000 grinded pellet with 2g NaN_3. Another negative control was prepared using 28 g of fish waste with 2g NaN_3.

A stirrer was inserted in each bottle and the bottles were put on the stirring platform. The bottles were kept in temperature adjustable water baths inside a cooling room to maintain the temperature 10 °C for 1st experiment and 15 °C for second experiment, throughout the time period. Before the experiment start, the volume of headspace in each chambers and volume of gas sensor chambers were measured and were checked for leakage of each chamber. During the test air is pumped from test chamber into the gas sensor chamber then again back to test chamber (Liebeg and Cutright, 1999). This used to calculate oxygen consumption in the test chambers. Here sample time interval was 4 hours and oxygen consumption were recorded as mg/h by a computer.

Two types of BOD tests were continued for 28 days for 1st experiment setups and 14 days for 2nd experiment setups for both OxiTop and micro oxymax. At the end TS, VS, TSS, VSS, TCOD, SCOD, TN, NH_4, NO_3, TP, PO_4, Ph of each bottles with two parallels without controls and blanks were done by using standards methods.

Table 3-4: Arrangement of the bottles for Micro-Oxymax BOD test 1

Chamber number	**Pellet/ Sample**	**Amount**
1	Fish waste 1	28 g
2	Fish waste 2	28 g
3	Fish waste 3	28 g
4	Fish waste 4	28 g
5	P 1000 pellet 1	0.6451 g
6	P 1000 pellet 2	0.5819 g
7	P 1000 pellet 3	0.6604 g
8	P 1000 grinded pellet 1	0.6451 g
9	P 1000 grinded pellet 2	0.5819 g
10	P 1000 grinded pellet 3	0.6604 g
11	P 1000 grinded autoclaved pellet 1	10 ml
12	P 1000 grinded autoclaved pellet 2	10 ml
13	P 1000 grinded autoclaved pellet 3	10 ml
14	Negative control 1	0.6293 g + NaN_3
15	Negative control 2	28 g + NaN_3
16	Positive control 1	Glucose
17	Positive control 2	Glucose
18	Blank 1	
19	Blank 2	
(+) ve control: 400 mg Glucose		
(-) ve control: P 1000 pellet + 2 g NaN_3		
Blank: 700 ml sea water		

3.6.4 Nutrient solution formulation

As mentioned in Table 3-5 nutrients were added using Nutrient A, B, C, D solutions. Nutrient solutions together were contained necessary nutrient for the enhancing the bioactivity as biocatelic substance (Liebeg and Cutright, 1999, Tabak et al., 1992). These nutrients were prepared separately in 1000 ml double distilled water and were sterilized by autoclaving before used (see Figure 3-3).

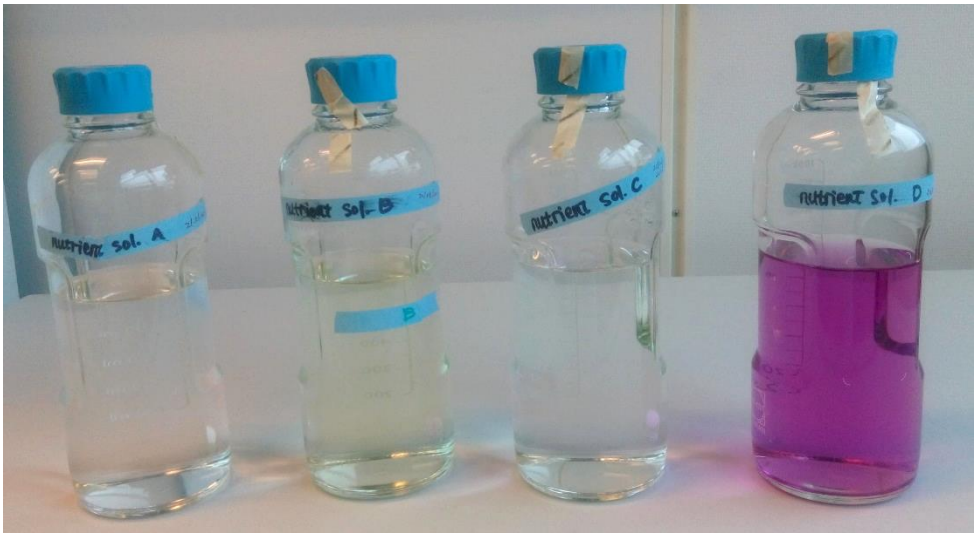

Figure 3-3: Nutrient solution prepared to be used in respirometer methods

Table 3-5: Composition of supplemental nutrient solution used in respirometer methods

Nutrient (g/L)	**Solution**			
	A	B	C	D
K_2HPO_4	16.2	NA	NA	NA
KH_2PO_4	0.8	NA	NA	NA
$NaNO_3$	NA	25	NA	NA
NH_4Cl	NA	0.6	NA	NA
$FeCl_3$	NA	0.05	NA	NA
EDTA	NA	0.2	NA	0.5
$CaCl_2$	NA	NA	2.5	NA
$MgSO_4$	NA	NA	1.5	NA
$MnSO_4.2H_2O$	NA	NA	NA	0.5
$MgSO_4.7H_2O$	NA	NA	NA	3
NaCl	NA	NA	NA	1
$FeSO_4.7H_2O$	NA	NA	NA	0.1
$CoCl_2.6\ H_2O$	NA	NA	NA	0.1
$CaCl_2.2H_2O$	NA	NA	NA	0.1
$ZnCl_2$	NA	NA	NA	0.1
$CuSO_4.5H_2O$	NA	NA	NA	0.01
$NiCl_2.6H_2O$	NA	NA	NA	0.02
Na_2SeO_3	NA	NA	NA	0.001
$AlK(SO_4)_2$	NA	NA	NA	0.01
H_3BO_3	NA	NA	NA	0.01
Na_2MoO_4	NA	NA	NA	0.01
$Na_2WO_4.2H_2O$	NA	NA	NA	0.01
** NA: Not added				

3.7 Testing settling velocity of pellets

For determining the settling velocity of the fish pellets four types of pellets were used. Before the test the diameter and length of each type of cylindrical pellets were tested using a venire caliper (precision 0.001 m). And weight of each pellet was measured using electrical balance (Mettler balance, model AJ 100; precision 0.1 mg).

Using a 150 cm length and 2.5 cm diameter Plexiglass settling column filled with cooled (5 °C) nonfiltered sea water using a funnel, each pellet was placed by some forceps in the center just below the water surface. A 10 cm and 1 m were marked and determined the time, each pellet passed 10 cm and 1 m lines using a stop watch. The sea water was completely renewed between each experiment to maintain the equal water properties (Law et al., 2014, Chen et al., 1999, Pérez et al., 2014, Vassallo et al., 2006, Bannister et al., 2016, Yandi and Kurtoğlu, 2016, Richard Moccia, 2007).

First and last 7.5 cm length of the column were ignored to get the time to start the timer manually and to remove the effect of bottom shear created by sedimentation column bottom on pellet velocity (Piedecausa et al., 2009, Chen et al., 1999). Settling velocity of five pellets in each type was determined in cold sea water (4 -5 °C).

At the end of the experiment weight of the pellet were determined using analytical balance (precision 0.1 mg) after absorbing additional water for 60 s into a absorbent paper (Piedecausa et al., 2009). Length and diameter were approximately measured using venire caliper (precision 0.001 m). Eventually the weight difference was expressed as percentage of weight difference to the initial weight.

3.8 Floating time

Measuring cylinders were filled with same amount of cold sea water (4 – 5 °C). Each fish pellet was kept on the sea water carefully using forceps. The floating times were calculated in each type of pellet (Vassallo et al., 2006).

3.9 Testing for fish pellets degradation

Three pellets from each pellet type (P 1000, P 500, P 200, P 80) were taken and put into each glass bottle filled with 200 mL sea water (4 -5 °C). and closed them. Glass bottles with sea water and pellets were kept in shaking table with low speed shaking and visually checked the degradation of pellets with time.

4 Results and discussion

This chapter contains the results of the experimental work and the discussion part of the results with current literature findings. Here try to explain the environmental effect comes all together with nutrients in pellets and fish waste, aerobic degradation, settling velocity of pellets and disaggregation of pellets.

4.1 Specific Theoretical Oxygen Demand calculation

Organic compound containing carbon, hydrogen, oxygen, and nitrogen convert to carbon dioxide, water, and ammonia in aerobic degradation. Oxygen need for this process called as carbonaceous theoretical oxygen demand.

During nitrification (see equation (2.6), (2.9) and (2.10)) ammonia convert to nitrite using oxygen. Oxygen requirement in both these processes together named as theoretical oxygen demand. Here we consider only carbonaceous theoretical oxygen demand. The oxygen demand for each compound as carbohydrate ($\gamma_{Carbohydrates}$), protein ($\gamma_{Protein}$) and fat (γ_{Fat}) called as specific theoretical oxygen demand.

Table 4-1: Average composition of fish feed pellets

Component	**Percentage (%)**
Protein	40
Fat	35
Carbohydrates	15
Moisture	8
Minerals	2

Here glucose formula ($C_6H_{12}O_6$) is considered for the calculation. Because at the end complex carbohydrates convert into their monomer units during degradation process and oxidize.

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6CO_2 + 6H_2O \tag{4.1}$$

$$\gamma_{\frac{O_2}{Glucose}} = \frac{6 * 32}{180} = 1.066\ \frac{gO_2}{g\ Glucose} \tag{4.2}$$

Therefore take,

$$\gamma_{Carbohydrates} = 1.1 \tag{4.3}$$

For this calculation the general protein structure considers as C_4H_6ON.

$$C_4H_6ON + \frac{17}{4}O_2 \rightarrow 4CO_2 + \frac{3}{2}H_2O + NH_3 \quad (4.4)$$

$$\gamma_{\frac{g\ O_2}{g\ Protein}} = \frac{\frac{17}{4} * 32}{84} = 1.61 \quad (4.5)$$

From C_4H_6ON and from bacterial cell structure formula $C_5H_7O_2N$ the nitrogen content of protein and bacterial cell originated. Specific theoretical oxygen demand for degradation of cellular structure (biomass) compound is less than this. Here this biomass part is not considered.

$$C_5H_7O_2N + 5\ O_2 \rightarrow 5CO_2 + 2H_2O + NH_3 \quad (4.6)$$

$$\gamma_{\frac{g\ O_2}{g\ cellular}} = \frac{5 * 32}{113} = 1.41 \quad (4.7)$$

Therefore take,

$$\gamma_{Protein} = 1.6 \quad (4.8)$$

Several fatty acids include in fish feed, saturated fatty acids as Myristic acids, Palmitic acid, Stearic acid and unsaturated fatty acid as linoleic acid, α-Linoleic acid and so on (see Table 2-3). Therefore, number of carbon in the formulas of the acids varies from 14 to 22. Here for the calculation C-17 is considered as representative fatty acid.

As main constituent of fat considering triglyceride molecule hydrolysis as in Figure 4-1 (Díaz et al., 2014).

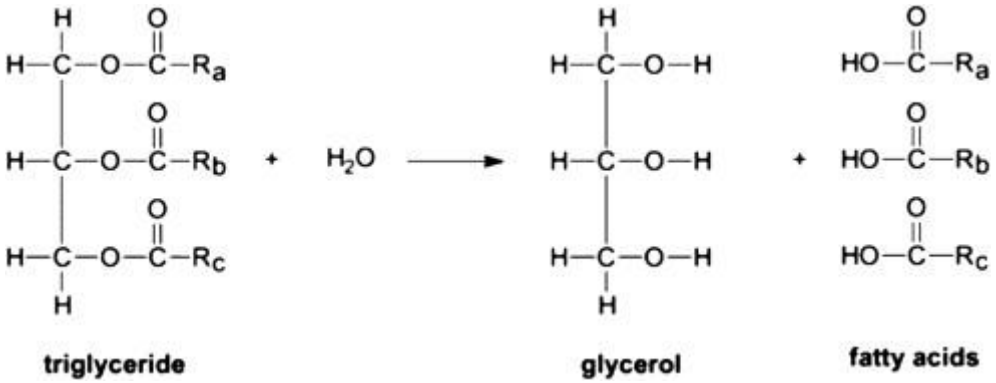

Figure 4-1: Hydrolysis triglyceride molecule

Considering three C-17 fatty acids the formula $C_{17}H_{34}O_2$ and glycerol ($C_3H_8O_3$) together gives $C_{54}H_{110}O_9$.By simplifying the numbers gives approximately formula as $C_6H_{12}O$.

Aerobic degradation of $C_6H_{12}O$,

$$C_6H_{12}O + \frac{17}{2}O_2 \rightarrow 6CO_2 + 6H_2O \quad (4.9)$$

$$\gamma_{\frac{g\,O_2}{g\,Fat}} = \frac{\frac{17}{2} * 32}{100} = 2.72 \tag{4.10}$$

Therefore take,

$$\gamma_{Fat} = 2.7 \tag{4.11}$$

This specific theoretical oxygen demand values use to calculate theoretical oxygen demand for fish pellets and fish waste containing fish feces.

4.2 ThOD calculation of pellets and fish waste

Even though TVS contain decomposition of some minerals as well, here assume as TVS represent only organic matter decomposition. As in equation (4.12) calculate the total carbonaceous oxygen demand for 1 g of TVS in the feed solution.

$$V_{O_2\ Fish\,feed} = 2.7 * 0.35 + 1.6 * 0.4 + 1.1 * 0.15 = 1.75\ g\ \frac{O_2}{g\ TVS} \tag{4.12}$$

$$TVS = TS - TFS = 92 - 5.24 = 86.76 \frac{g}{100g} = 868\ mg/g \tag{4.13}$$

Finally, Theoretical oxygen demand in 1 g of feed is calculated as equation (4.14) by multiplying with TVS % in feed solution.

$$ThOD = V_{O_2\ Fish\,feed} * \text{TVS \%} = 1.75\ g\ \frac{O_2}{g\ TVS} * 0.868\ \text{g TVS/g pellet}$$

$$ThOD = 1.52\ g\ \frac{O_2}{g\ pellet} \tag{4.14}$$

Table 4-2: Theoretical oxygen demand for each pellet calculation

Pellet	Weight (g)	ThOD (Theoretical Oxygen Demand) (g O_2)	
P1000	0.66	1.00 (1.52 * 0.66)	Micro-Oxymax method
P500	0.41	0.62 (1.52*0.41)	
P200	0.093	0.14 (1.52 * 0.093)	OxiTop method
P80	0.058	0.09 (1.52*0.058)	

From ThOD of each pellet give an idea about the oxygen demand of pellets. Pellets having high ThOD (P 1000 and P 500) used in Micro-Oxymax (have more oxygen supply) and lower ThOD values (P 200 and P 80) used in OxiTop (have limited oxygen amount) for BOD test. During Micro-Oxymax test headspace gas continuously renew (dynamic respirometry system) and in OxiTop have static respirometry system.

According to Table 2-2;

$$V_{O_2\ Fish\ waste} = 2.7 * 0.11 + 1.6 * 0.26 + 1.1 * 0.36 = 1.11\ g\ \frac{O_2}{g\ TVS} = 1110\ \frac{mgO_2}{g\ TVS} \quad (4.15)$$

Assume ThOD for fish waste is like P 1000 pellet means, about 1g O_2 in chamber with sea water. Therefore, fish waste also used in Micro- Oxymax method.

$$ThOD = \frac{1\ \mathrm{g}\ O_2}{0.7\ l} = 1429\frac{mgO_2}{l} \quad (4.16)$$

$$TVS = \frac{ThOD}{V_{O_2\ Fish\ waste}} = \frac{1429\frac{mgO_2}{l}}{1110\ \frac{mgO_2}{g\ TVS}} = 1.29\ g\ TVS/l \quad (4.17)$$

$$Amount\ of\ fish\ waste\ in\ 0.7\ ml = m_{fish\ waste} \quad (4.18)$$

$$TVS\ in\ fish\ waste\ (Table\ 4-4) = 4.56g\frac{TVS}{100g\ fish\ waste} \quad (4.19)$$

$$m_{fish\ waste} = \frac{1.29\frac{g\ TVS}{l} * 0.7l}{4.56g\frac{TVS}{100g\ fish\ waste}} = 20\ g\ wish\ waste \quad (4.20)$$

Form above calculations determine the ThOD of each fish pellets and fish waste to determine the suitable method use for BOD test. Further these ThOD values are used to get remaining organic matter content in the solution after BOD test to determine the degree of aerobic degradation in marine condition.

4.3 Preparation of the autoclaved solution for grinded P 1000 and P 200 pellets

$$Volume\ of\ autoclaved\ solution\ in\ the\ preparation\ bottle = 100ml$$

$$Volume\ of\ autoclaved\ solution\ added\ to\ chamber\ bottle = 10ml$$

Therefore, 1g ThOD should be in 10 ml added autoclaved solution to represent P 1000 pellet (Table 4-2).

$$Concentration\ of\ COD\ added\ by\ P\ 1000\ grinded\ pellet = \frac{1\ g\ COD}{10\ ml} = 100\ \frac{g\ COD}{l} \quad (4.21)$$

Therefore,

$$In\ 100\ ml\ amount\ of\ COD\ present = \ 10g\ COD \tag{4.22}$$

$$Number\ of\ \ grinded\ P\ 1000\ pellets\ needed\ to\ get\ 10g\ COD\ in\ 100\ ml\ sea\ wa = 10 \tag{4.23}$$

$$Concentration\ of\ COD\ added\ by\ P\ 200\ grinded\ pellet = \frac{141.4\ mg\ COD}{10\ ml} = 14.14\ \frac{mg\ COD}{l} \tag{4.24}$$

Therefore,

$$In\ 100\ ml\ amount\ of\ COD\ present = \ 1.41\ mg\ COD \tag{4.25}$$

$$Number\ of\ \ grinded\ P\ 200\ pellets\ needed\ to\ get\ \ 1.41\ mg\ COD\ in\ 100\ ml\ sea\ water = 10 \tag{4.26}$$

From this calculation determine the volume of autoclaved solution add into the chambers. Autoclavation is used to do the hydrolysis process using heat to make it easy for biodegradation. Because sometimes water temperature, pellet storage temperature, physical properties of pellets affect for aerobic degradation in marine system. Here try to see the difference of kinetics and degree of biodegradation in autoclaved sample compare with whole pellets. This calculation results used in sub topic 4.14.

4.4 Mass balance system for sea water volume determination of BOD test

The volume of sea water need for micro OxiTop decide using mass balance system as follows;

$Density\ of\ air\ \ at\ 10\ °C = 1.247\ kg/m^3$

$Volume\ of\ the\ chamber = 0.001\ m^3$

$Oxygen\ percentage\ in\ air\ \ = 21\%$

$$Amount\ of\ oxygen\ in\ 1L\ bottle = 1.247\ \frac{kg}{m^3} * 0.001 m^3 * 0.21 = 0.262 g \tag{4.27}$$

$$Average\ \ ThOD\ for\ P\ 200\ and\ P\ 80 = \frac{0.1414 + 0.0882}{2} = 0.1148 g \tag{4.28}$$

$$Oxygen\ weight\ ratio\ in\ bottle\ and\ average\ ThOD = \frac{0.1148\ g}{0.262\ g} = 0.438 \tag{4.29}$$

So, 600 mL of head space and 400 mL volume of sea water were selected for each chamber in OxiTop.

$Oxygen\ solubility\ of\ sea\ water\ at\ 10\ °C = 8\ mg/L$ (ToolBox, 2005)

$$Oxygen\ \text{solubility of 400 mL sea water at 10 °C} = 8\frac{\text{mg}}{\text{L}} * 0.4\ \text{L} = 3.2\ \text{mg} \quad (4.30)$$

Therefore, the headspace volume is theoretically sufficient for the aerobic degradation process.

Because Micro-Oxymax is a dynamic respirometry system, continuously maintain the Oxygen percentage of air as 21 %. Therefore, 700 mL volume of sea water selects in each chamber for fully dissolution of P 1000 and P 500 pellets which are containing high fat amount and higher weights (see Appendix C).

Four parallels of each pellet were in both in Micro-Oxymax and OxiTop methods to reduce the error readings and increase the precision of the results.

4.5 Positive control, Negative control and blank in Micro-Oxymax and OxiTop systems

The reason for using positive control to assess the method is working properly, whether it is reproducible, and it have enough sensitivity to get the known results. Glucose, Sodium acetate, Sodium benzoate, aniline readily biodegradable compounds, can use as positive reference for the biodegradation related method (Comber and Holt, 2010). Here 400 mg of glucose and 100 mg of glucose in sea water used as positive control amount is determined as follows;

$$\gamma_{\frac{O_2}{Glucose}} = 1.066\ \frac{gO_2}{g\ Glucose} \quad (4.31)$$

Therefore,

$$Amount\ of\ oxygen\ need\ to\ biodegrade\ 0.4\ g\ of\ glucose\ theoritically = 1.066 * 0.4 = 0.4264\ g \quad (4.32)$$

$$Amount\ of\ oxygen\ need\ to\ biodegrade\ 0.1\ g\ of\ glucose\ theoritically = 1.066 * 0.1 = 0.1066\ g \quad (4.33)$$

These values are representing the ThOD for both P 1000, P 500 and ThOD for both P 200 and P 80 respectively.

For some biodegradability test instead of using highly soluble substances poorly soluble substances used as positive control as disooctylphthalate or anthraquinone (Comber and Holt, 2010).

Negative control used to get the oxygen utilization value for degrade other biodegradable chemicals present in pellet with sea water solution. It is not give any negative or positive effect to the solution as name suggested. Normally negative control is treated with the same solutions or buffer as other test solution chambers. Hexachlorobenzene, Benzo(a)pyrene and Hexachllrohexane are some difficult biodegradable example compounds for negative control reference chemical which can use in biodegradation tests (Comber and Holt, 2010). In this test sodium azide (NaN_3) was added to negative control with P 500 and P 80 pellet in OxiTop and Micro-Oxymax tests respectively as toxic to destroy microorganisms involve in biodegradation.

Blank is normally media or inoculums without testing compound (Comber and Holt, 2010). In this experiment used sea water with nutrients without pellets to check the background oxidation is sea water.

Therefore, these blanks, negative controls and positive controls are used to determine the degree of aerobic degradation in pellets and fish waste themselves in marine environment using a reproducible, precise method.

4.6 COD value and other compound results in analysis of fish pellet sample before BOD test

Table 4-3: Chemical Analysis results of fish feeds solution

Test	Range(mg/L)	Value	% (W/W)
Original Solution:			
TCOD (Hg free)	100 - 1500	6025 ± 955 mg/L	60.7
NH_4	0.5 - 16	16.1 ± 0.8 mg/L	0.16
TP	0.05 - 5	56 ± 12 mg/L	0.56
TN	0.5 - 15	24 ± 0 mg/L	0.24
PO_4	0.05 - 5	40 ± 0 mg/L	0.40
NO_3	0.5 - 18	6.5 ± 0.5 mg/L	0.07
TS	-	92 ± 0.15 g/100 g	92
TFS	-	5.24 ± 0.41 g/100 g	5.24
VS	-	86.8 g/100 g	86.8
Filtrate:			
SCOD (Hg free)	100 - 1500	1240 ± 60.5 mg/L	12.5
NH_4	0.5 - 16	13.1 ± 1.9 mg/L	0.13
TP	0.05 - 5	43 ± 9 mg/L	0.44
TN	0.5 - 15	16 ± 0 mg/L	0.16
PO_4	0.05 - 5	39 ± 0 mg/L	0.39
NO_3	0.5 - 18	2 ± 0 mg/L	0.02

Aerobic bacteria utilize oxygen and degrade organic matter in the solution through respiration process. Microorganisms produce energy through respiration for living and produce cells.

Theoretical oxygen demand for this process for degrade carbohydrates, protein, fat can be calculated as CTOD. CTOD vales for pellets as Table 4-2. This value and measured COD value for P 1000 pellet should be near values.

When consider P 1000 CTOD value, expected COD value as follows;

$$CTOD\ of\ for\ 1\ g\ \ pellet = 1.52\ g\ \frac{O_2}{g\ pellet} \tag{4.34}$$

$$Weight\ of\ the\ pellet\ in\ the\ solution\ made\ for\ analysis = 4.96\ g \tag{4.35}$$

$$Expected\ COD\ or\ CTOD\ for\ 500\ mL\ solution\ with\ 4.96\ g = 1.52 * 4.96 = 7.54\ gO_2 \tag{4.36}$$

$$Expected\ COD\ or\ CTOD\ for\ 1000\ mL\ solution = \ 7.54 * 2 = 15.08\ gO_2 = 15080\ mgO_2 \tag{4.37}$$

This value is more than double than measured COD value 6025 mg/L (see Table 4-3). This is because the solution contains high amount of fat bubbles when mixing with distilled water. The fat appeared in the top as a separate layer (see Figure 4-2).

For the analysis to get the representative sample the volume was taken from the middle layer. Therefore, most of this fat layer not present in the volume taken for the analysis. According to the composition pellets contain 35% of fat (Table 4-1). Therefore, COD for most of fat is missing in this value. Not only COD other compounds as ammonia, phosphate, nitrate which combined with fat compounds and undissolved particles are not included in the measurements and give lower values. Therefore, other test results as total nitrogen, total phosphorous, and nitrate and ammonia content also low in this analysis.

Figure 4-2: Fat layer floating on the solution

Soluble chemical oxygen demand value (SCOD) is lower here, because of the similar effect from fat layer and due to lots of particles in the solution. There were some particles in the solution and it was not a clear solution even after mixed well (see Figure 3-1). Even though SCOD content is lower in big amount compare with TCOD the ammonia, nitrate, total nitrogen, phosphate, and total phosphorous content is lower in small amount and it is reliable compare results with before filtration. This is suggesting with filtration reliable amount of these compound come into the solution.

According to the results mostly total nitrogen contain as nitrate and ammonia. Total phosphorous contain mostly as phosphate in the solution (see Table 4-3). Total phosphorous (TP) content is higher in fish feed than total nitrogen (TN) according to the measured values. Percentage values of each compound according to the weight used for preparing the solution is mentioned in Table 4-3. Each percentage values are lesser than normally present values as percentage in fish feed. As example the percentage of phosphorous content is about 1.2% in Atlantic salmon fish feed (Reid et al., 2009), but here it is about 0.56% (w/w%).

Even though COD value should be about 2.5 times higher than measured value, as above by taking difference of TS and TFS values (using TVS) theoretically initial COD value (CTOD) can be calculated. Because for measuring TS and TFS values crushed pellets put into oven without using the pellet dissolved solution. So, by that can get an idea about the COD of the pellet before doing the BOD test.

4.7 Comparison of fish pellet and fish sludge composition

Table 4-4: Experimental results of Fish sludge sample

Test	Range (mg/L)	Values
TCOD (Hg free)	100 – 1500	60239 ± 1744 mg/L
SCOD (Hg free)	100 – 1500	15600 ± 250 mg/L
NH_4	0.5 - 16	185 ± 4 mg/L
TP	0.05 – 5	911 ± 3 mg/L
TN	0.5-15	2088 ± 165 mg/L
PO_4	0.05 – 5	416 ± 10 mg/L
NO_3	0.5-18	140 ± 0 mg/L
TS	-	5.9 ± 0.15 g/100 g
TFS	-	1.32 ± 0.03 g/100 g
TVS	-	4.56 ± 0 g/100 g
Filtrate:		
NH_4	0.5-16	1.40 ± 0 mg/L
TP	0.05 – 5	4.42 ± 0.07 mg/L
TN	0.5-15	> 15 mg/L
PO_4	0.05 – 5	4.20 ± 0.02 mg/L

Mostly phosphorus difficult to digest in the salmon fish body and excrete via feces. Specially to digest plant origin phosphorous difficult to digest due to lack of phytase enzyme (Wang et al., 2012, Wang et al., 2013). The main way to remove phosphorous from the fish body is through feces. Therefore, high amount of phosphorous appear in the fish sludge sample mostly contain fish waste sample (see Table 4-4). About 85% digestibility of protein release high amount of nitrogen via feces (Cheshuk et al., 2003) and (Mente et al., 2006).

This is the reason for higher ammonia in sludge sample and some ammonia convert to nitrate through nitrification process (see Figure 2-1). The total solid content, total fixed solid content is lower in fish sludge sample than fish pellet sample. High TCOD content suggest that high amount of organic matter in the sludge sample. Further by dichromate in the COD cells can oxidize some inorganic substances and help to increase the organic content of the sample and increase the COD measurement value. However according to the calculations (see equation (C.1)) even though the organic matter content is low, feces percentage in the solution is very high in sludge sample. It is a very thick concentrated solution. This gives high amount of measured COD value in fish sludge sample.

These analyses are done to know the amounts of TN, ammonia, TP, phosphate, organic compounds come to the marine environment with fish pellets (section 4.7) and fish waste. These results can use to compare the results with chemical analysis of pellets solutions and fish waste solutions after BOD test to know how much of TN, ammonia, TP, phosphate, organic compounds removed from the marine environment with aerobic degradation of organic matters in fish waste and pellets by bacteria without suppling nutrient for the negative environmental effect as eutrophication. Further initial oxygen demands in fish feed and waste taken from TVS, TCOD values are used to determine the kinetics and degree of aerobic degradation in marine system.

4.8 Biodegradation of organic matter and Conversion of organic matter into bacterial biomass

With time used oxygen rate increase initially due to high biodegrading of organic matter. Organic compound act as electron donor (equation (4.39)) and oxygen act as electron acceptor (equation (4.38)) in energy production process (equation (2.5)).

$$\frac{1}{4}O_2 + H^+ + e \rightarrow \frac{1}{2}H_2O \tag{4.38}$$

Fish body used mostly protein as energy source.

$$\frac{1}{d}C_nH_aO_bN_c + \frac{2n-b+c}{d}H_2O \rightarrow \frac{n-c}{d}CO_2 + \frac{c}{d}NH_4^+ + \frac{c}{d}HCO_3^- + H^+ + e^- \tag{4.39}$$

Overall equation is given in equation (2.5).

If only this energy production part occurs, it shows same reaction as complete degradation of organic compounds (equation (2.5)). But, some amount of organic matter converts into bacterial biomass. This is called as bacterial yield. In this scenario, bacteria use organic matter for cell synthesis using organic compound as electron donor as in energy production electron donor (equation (4.39)). Part of this electrons supply energy to other electrons with electron acceptor (equation (4.40)) converts into microbial biomass (equation (4.41)).

$$\frac{1}{5}CO_2 + \frac{1}{20}NH_4^+ + \frac{1}{20}HCO_3^- + H^+ + e \rightarrow \frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O \quad (4.40)$$

Ammonia (NH_4^+) gives nitrogen to produce bacterial cell structure formula $C_5H_7O_2N$ (equation (4.40)) and for nucleic acid production. If this $C_5H_7O_2N$ oxidize as equation (2.5), BOD value become same as COD value. But naturally it occurs parallel with cell synthesis as in equation (4.41) (Tchobanoglous et al., 2002).

$$\frac{1}{d}C_nH_aO_bN_c \rightarrow \frac{1}{20}C_5H_7O_2N + \left(\frac{n-c}{d} - \frac{1}{5}\right)CO_2 + \left(\frac{c}{d} - \frac{1}{20}\right)NH_4^+ + \left(\frac{c}{d} - \frac{1}{20}\right)HCO_3^- + \frac{9d - 20(2n - b + c)}{20d}H_2O \quad (4.41)$$

Finally, these two steps, energy production and cell synthesis called bacterial growth.

The energy reduction due to biomass synthesis (equation (4.41)) is one reason for not measuring similar level of oxygen demand as COD in BOD values.

Organic matter comes from uneaten fish feed and feces mainly decide the oxygen utilization amount by microorganisms in aquaculture system. If there are high amount of organic matter, it cause excess utilization of oxygen usage for degradation occurring hypoxia condition in water (Klanjšček et al., 2012). For half of benthic fauna, if the oxygen amount is lower than about $3mgO_2L^{-1}$, can leads lethally harmful condition to them. Therefore, here oxygen utilization of biologically degrade each pellet type studies are important to see the impact to oxygen in water and study the fate of the uneaten pellets in water. Further above energy production and cell synthesis equations use to explain the cumulative and rate figures of usage of oxygen by bacteria in fish pellets and fish wastes BOD tests and this condition is aerobic degradation process of fish pellets and feces in marine fjord water.

4.9 Micro-Oxymax results explanation with graphs

In Micro- Oxymax measurement points were taken 4 hours' time interval. The oxygen utilization rate for P 1000 and P 500 (see Figure 4-3 and Figure 4-5) are very low in the beginning, because degradation starts after some time. Pellets absorb water and slowly start to degrade. It will take about one day for pellet to fully absorb water and get rid from tough pellet nature. Due to agglomeration and cohesive effect of pellet particles, it appears as pellet even after it detach into particles. It is like agglomerated particle ball. With time microorganisms starts to biologically degrade the organic matters in the pellet using oxygen. Agitator used in each bottle help to dissolve the agglomerated particle with water to making it easy to biodegrade by microorganisms.

With increasing the energy production of microbes using organic matter as equation (4.41), cell synthesis increases the microbial population using some amount of energy produces through aerobic reparation. At the beginning microorganisms needs increasingly energy to make the population increase stable condition. The log phase of microbial growth occurs at this part. This increases the oxygen utilization rate of microbes. This is the reason for increasing rate from 0.4 mg/h/ml to about 1.6 mg/h/ml of oxygen utilization from 2 to 4 days in Figure 4-3 and from 0.4 mg/h/ml to about 1.4 mg/h/ml from 2 to 3 days in Figure 4-5. In cumulative figures oxygen utilization increase up to 100 mg $O_2$for P 1000 and 60 mg $O_2$for P 500.

Then 4-12 days Figure 4-3 and 6-12 days (Figure 4-5) the utilization rate of oxygen becomes constant (about 1.4 mg/h/ml in Figure 4-3 and about 1.3 mg/h/ml in Figure 4-5). At this moment cell synthesis, energy productions via aerobic respiration in the microbial population are

tending to become equilibrium stage and use oxygen less rate than beginning to maintain microbial life. This is representing by same time gap in Figure 4-4 and Figure 4-6. The cumulative graph tent to become constant at this movement. Cumulative oxygen utilization increase averagely from 100 mg O2 to 400 mg O2 for P 1000, 1.P1000, 3, P 1000, 4 in Figure 4-4 and averagely from 100 mg O2 to 300 mg O2 for P 500, 2, P 500,3 and P 500, 4 in Figure 4-6. P 1000, 2 and P 500, 1 show lower values compare with other pellets in same range due to lower weight.

With the time when the organic matter content reduces the energy source for microorganisms becomes lower and energy production go down. Further microorganisms start to death due to lack of energy and production of new cells (biomass) also become lower. With lowering amount of organic matter, the oxygen utilization rate reduces in 13 -28 days in Figure 4-3, 11-28 days in Figure 4-5. And less increasing rate of Figure 4-4 and Figure 4-6 during same time gaps simultaneously. Rate reduces from about 1.4 mg/h/ml to 0.5 mg/h/ml averagely in Figure 4-3 and 1.3 mg/h/ml to 0.2 mg/h/ml in Figure 4-5. During same time gap in cumulative graphs value increment occur from 400 mg O_2 to 800 mg O_2 with lower rate in Figure 4-4 averagely for P 1000, 1.P1000, 3, P 1000, 4 and 300 mg O_2 to 450 mg O_2 increment averagely in lower rate for 500,2 ,P 500,3 and P 500, 4 in Figure 4-6. But still there are enough organic matters in the solution to produce energy for microbes to maintain the population with less percentage of cell death than biomass synthesis. Only energy production rate is going down.

Constant level about 0.5 mg/h/ml in Figure 4-3 and about 0.2 mg/h/ml in Figure 4-5 are going to occur due to equilibrium stage of the system after 28 day and oxygen utilization rate also going down further in Figure 4-3 and Figure 4-5. At this movement with energy production via aerobic organic matter degradation, cell synthesis, cell deaths are at equilibrium condition. This is the optimum level of BOD can be seen in this system (about 800 mg O_2 in Figure 4-4 and about 450 mg O_2 in Figure 4-6). Here organic matter degradation is at the optimum level with the optimum microbial population in the system.

This constant condition will maintain for some hours and then organic matter content in system further reduces and energy production reduces. Then there is not enough energy for synthesis of cell and microbial death is increase. Therefore, microbial population can't be stable anymore. The oxygen utilization rate Figure 4-3 and Figure 4-5 goes down and also cumulative utilization of oxygen goes down (Figure 4-4 and Figure 4-6). This is not shows in Figure 4-3, Figure 4-5 and Figure 4-4, Figure 4-6.

Same scenario happens in solution with P 1000 and P 500 as above description with changes of values.

Even though the same pellet type used four parallels of P 1000 graphs are not overlapping each other. Four pellets only give same pattern graphs. The P 1000, 2 gives lower oxygen utilization rate at the beginning and middle (Figure 4-3). This can be explained with Table 3-3.The weight of P 1000,2 is lower than other pellets. Therefore, contain lesser organic matter than other pellets. The weights of pellets are varying as $W_{P1000,4}$>$W_{P1000,1}$>$W_{P1000,3}$>$W_{P1000,2}$ in this experiment. Organic matter content is also following same pattern as weights and therefore the graphs give higher oxygen utilization value for higher organic matter content solution to degrade them. The difference is very clear in cumulative oxygen utilization graphs (Figure 4-4).

In Figure 4-3 the rate of utilizing oxygen graphs overlap with each other after utilizing higher portion of biodegradable organic matter in the system optimistic way. Then the solutions remain mostly lesser amount of biodegradable organic matters, unbiodegradable organic matters, death microbial cell and so on. At this movement oxygen consumption rate in four

chambers more or less the same due to same fewer amounts of organic matter contents. But in cumulative graphs shows total oxygen consumption from the beginning to end, it shows clearly the lines for each pellet (Figure 4-4).

When consider about the weight difference of each pellet, P,1000,2 shows high difference with other three pellets comparatively. Therefore, after soaking the pellet in sea water and after the biological degradation start, cumulative oxygen utilization graph of P 1000,2 clearly separates from other graphs (Figure 4-4) due to lower organic matter content. The weight difference between P 1000,4 and P 1000,1 is lower than weight difference between other pellets. Therefore graphs lines related to P 1000,4 and P 1000,1 goes closely in both Figure 4-3 and Figure 4-4 due to less difference in organic matter content. In Figure 4-4 at the end these two lines overlap together showing the similarity of amount of organic matter content.

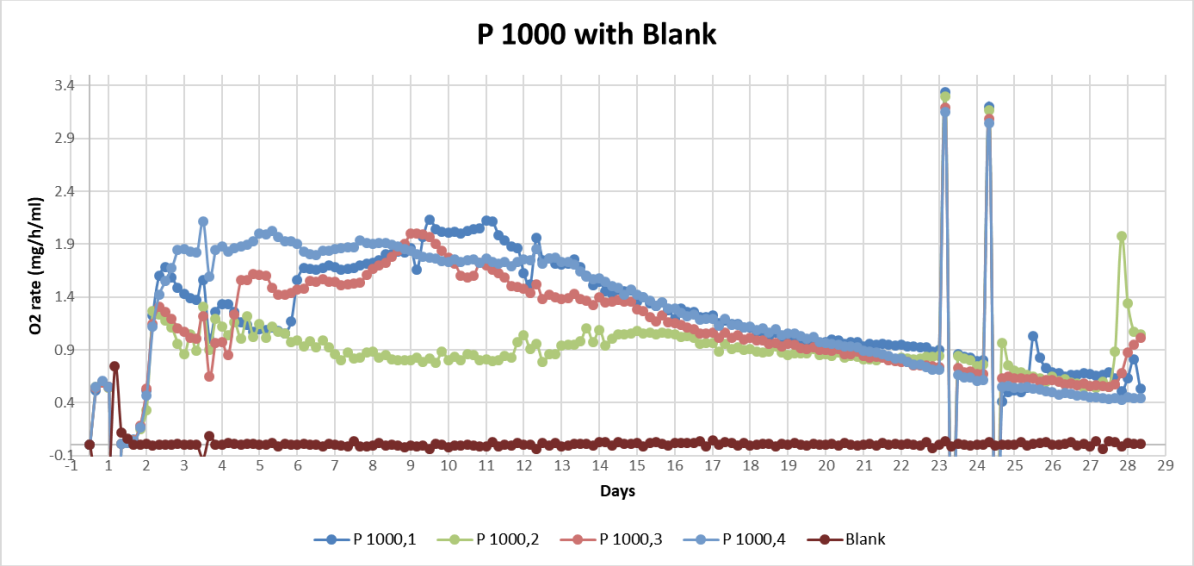

Figure 4-3: O_2 utilization rate of P 1000 with blank

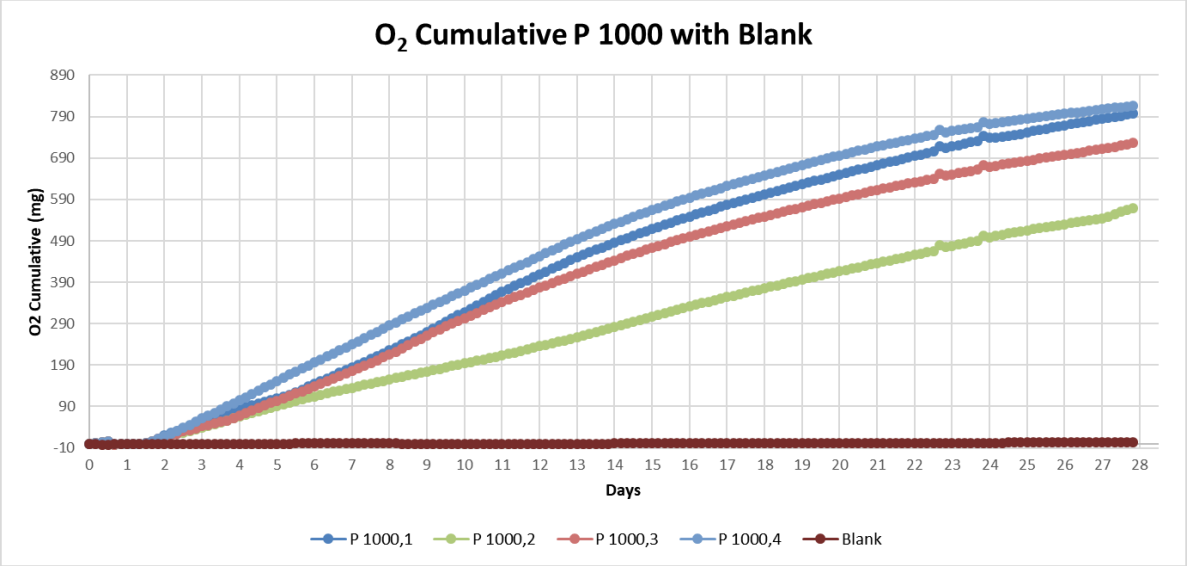

Figure 4-4: Cumulative O_2 utilization of P 1000 with blank

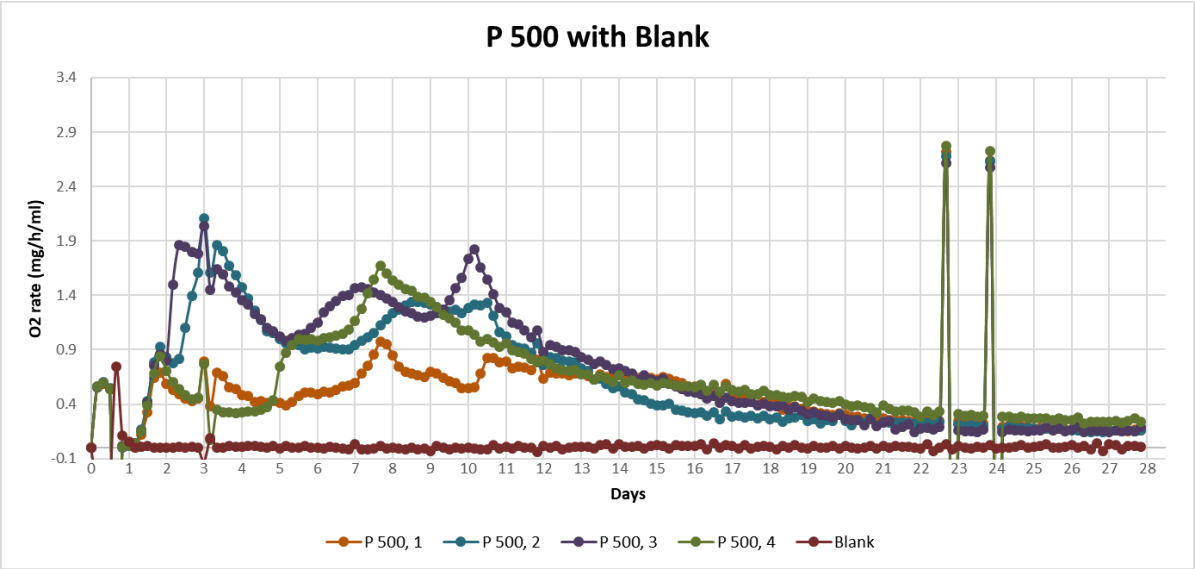

Figure 4-5: O_2 utilization rate of P500 with blank

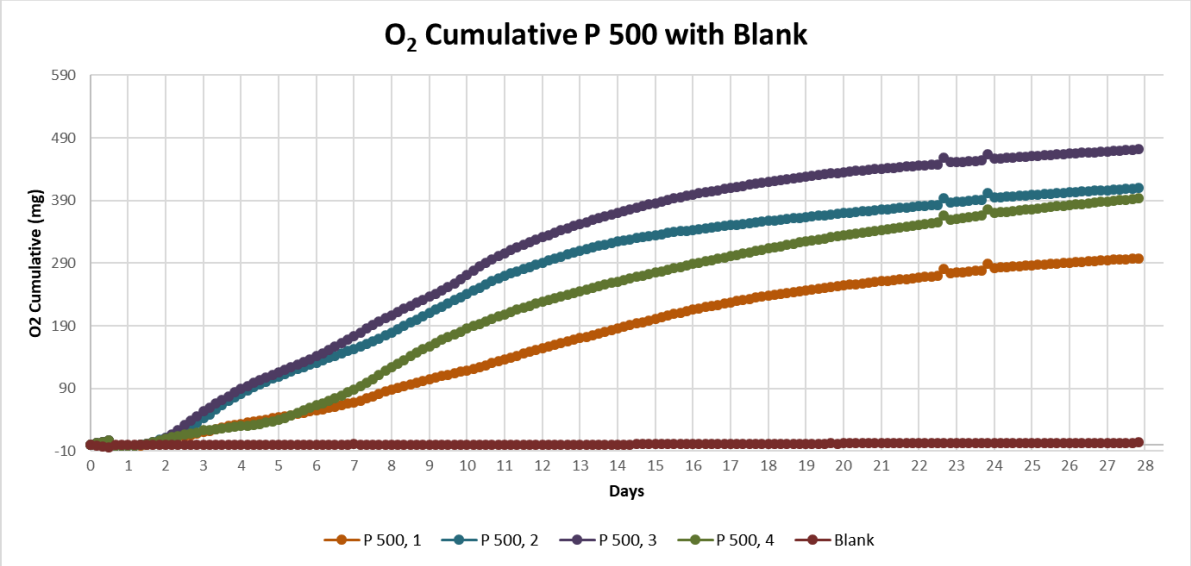

Figure 4-6: Cumulative O_2 utilization of P500 with blank

Similarly, for P 500 pellets weights are vary as $W_{P\ 500,3}$>$W_{P500,2}$>$W_{P500,4}$>$W_{P500,1}$.The weight difference between each pellet of P 500 shows the difference of the gap between each pellet. As example weight difference between $W_{P\ 500,3}$ and $W_{P500,2}$ is lesser than weight difference of $W_{P\ 500,3}$ with other two pellets. So during the first part of the Figure 4-5 and Figure 4-6, P 500,3 and P 500,2 overlapped and same happen with P 500,4 and P 500,1. P 500,4 and P500,2 again overlap at the end part of Figure 4-6 due to achieving similar amount of organic content oxidation at the end. After initial part the P 500,1 can clearly shows separate graph line with other three pellets due to lower weight of the pellet compare with other three pellets in Figure 4-6. In Figure 4-5 at the end part graphs overlap each other due to remaining same amount of organic matter after optimum oxidation. At the middle part of Figure 4-5 the line for P 500,3 always shows high oxygen utilization rate due to high organic matter amount than other pellets.

Two instrumental errors due to pressure increment which are occur at before and after 23-24 days. This pressure increment shows in Appendix E. It shows in oxygen utilization rate and cumulative graphs for P 1000 and P 500 as two sudden increments. This can be clearly seen in oxygen utilization rate graphs (Figure 4-3 and Figure 4-5) and also in Appendix E.

$$PV = nRT \tag{4.42}$$

Because according to the ideal gas law (equation (4.42), assuming the gas volume (V) and Temperature (T) is constant; pressure is directly proportional to the mole amount of the air. Therefore, with the pressure increment the moles of the air should be increase (equation (4.43)).

$$P \propto n \tag{4.43}$$

In this case this explains how the oxygen rate comes to the chamber and oxygen cumulative values increment. This is not because of organic matter increment in the chamber, this is because of sudden pressure increment in chambers.

All the figures for P 1000 and P 500 are shows with the blank line. Figures for P 1000 and P 500 shows there is very lower background oxidation in chambers. Because all the time the value is near to zero line.

In the real scenario the full aerobic biodegradation is not occur. Half of the organic matter can be degraded aerobically by using available oxygen in marine fjord water and rest can be degraded anaerobically causing worst environmental damages as odor development. It depends on the oxygen availability of water. By taking average ultimate BOD of P 1000 and P 500 as 800 mg and 450 mg in 700 mL sea water (Figure 4-4, Figure 4-6), the half of the organic matter biodegrade $t_{1/2}$ at day 11-13 in P 1000 and days 9-12 in P 500 (Figure 4-4, Figure 4-6). By analyzing BOD graphs of Micro Oxymax can get an idea about the way of aerobic degradation process with oxygen availability and time needed to biodegrade uneaten fish pellets or parts in marine fjord system. Cumulative graphs can used to determine the degree and kinetics of aerobic degradation in marine fjord system (section 4.10).

4.10 Difference between ThOD and BOD values in P 1000 and P 500 solutions

When consider about the highest cumulative graph value for P1000,4, the value is 816 mg O_2 . For P 500,3 the final value is 472 mg O_2. Theoretically calculated value using TVS for P 1000 pellet is 1003 mg O_2 and for P 500 pellet, it is 623 mg O_2 (Table 4-3).

The difference of biological oxygen demand (BOD) and ThOD (consider same as COD here) for P 1000 is 187 mg O_2 for P 500 the difference is 151 mg O_2.This difference is because energy usage for convert part of organic matter in to biomass (cell synthesis) as describe above and also low biodegradable compounds in the solution. Some compound can be able to biodegradable chemically but difficult to do biologically. Furthermore Figure 4-7 shows the final appearance of P 1000, 4 and P 1000,1 pellets containing chamber. In both P 1000 and P 500 pellets containing chambers there are fat bubbles in the top of the water layer. Because P 1000, 4 and P 1000,1 contain higher weight (high organic matter) the fat is appear as shown in Figure 4-7. Throughout the experiment the size of the fat layer reduces slowly, because biodegradation can be done only by surface bacteria. Therefore after 28 days of the experiment still there is fat remaining on the top without degradation. Fat is organic compound which

contain in especially, in P 1000 and P 500 pellets in high percentage. Therefore, oxygen utilization for degrade this fat amount remain in the solution after 28 days BOD experiment is not present in final cumulative oxygen utilization value. This is also one of main reason in this experiment for the difference between ThOD value for the P 1000, P500 pellet and final cumulative oxygen value after BOD. Not only fat small amount of the particles from the pellets remain in chambers without dissolving in all chambers (Figure 4-8). Oxygen utilization values for biodegradation of these particles are also missing in the cumulative final value.

Figure 4-7: P 1000,4 and P 1000,1 containing chamber

Figure 4-8:Particle presents in chambers after BOD

Therefore, from this difference between maximum cumulative BOD and calculated ThOD values before BOD test, can get an idea about the degree of aerobic biodegradation of uneaten fish pellets in fjord fish farms after 28 days if enough oxygen is available. By taking the ratio of maximum cumulative oxygen utilization to ThOD value (Table 4-2) the degree of aerobic degradation for P 1000 pellet is about 81 % and for P 500 pellet is about 76%.

4.11 Positive control graphs and negative control graphs from Micro-Oxymax

The pattern Oxygen utilization rate of positive control (Appendix E) and cumulative oxygen utilization graph (Figure 4-9) is mostly similar to P 1000 and P 500 figures. Therefore, positive control figures suggest the method is working properly. Both parallels of positive controls give more or less same values and lines overlap with each other most parts in both figures and specially in Figure 4-9. In rate graph around day 3 (at 20) there is a peak oxygen utilization due to hydrolysis of simple compounds and again there is a substantial growth around day 13 (at

80). The reason for this due to start hydrolyzing of complex compounds as protein after hydrolysis simple compounds. This is clearly seen in rate graph than Figure 4-9.

The last value in Figure 4-9 is about 430 mg O_2. Theoretically calculated value (ThOD) is 426 mgO_2 (equation (4.32)). The difference between these values is 4 mgO_2.This suggest the difficult biodegradable compounds are very low in glucose, because it is easily fully biodegradable compound and further biomass yield is lower in glucose than pellet solution. Eventually the glucose solution shows the method gives reproducible and sensitive enough to give accurate results.

P 500 pellet use for negative control one was not dissolved with the solution even with an agitator to mix pellet with water. But it is not good because purpose of using negative control is to determine the oxygen utilization of other biodegradable chemicals in the solution with pellets. Here assume microorganisms are destroyed by NaN_3. Figure 4-9, Figure 4-9 shows the graphs of negative control for oxygen utilization rate and cumulative oxygen utilization. In these graphs also, the error readings due to high pressure increments can be seen clearly. The oxygen utilization rate graph (Figure 4-9) is overlapping and show value near to zero. But in cumulative graph (Figure 4-9) shows deviated graphs from each other after day 3. This is because the pellet in negative control 1 is not dissolved with water (green line). The pellet in negative control 1 produce some gas bubbles due to air trapping inside the pellet. This gas bubbles production is identified by machine as pressure increment in headspace of chamber and reduce oxygen utilization rate (Figure 4-9). That is why it gives negative values for negative control 1. But it is small deviation. In negative control also have cumulative oxygen utilization value from beginning to end value around 3 mgO_2 in negative control 2. Negative control 1 gives negative value -3 mgO_2 at the end. Therefore, pellet dissolution has affected for the values. To get figures for P 1000 and P 500 the negative control value is reduce from each point to remove the oxygen utilization for biodegradation of chemical matter in solution with pellets. Here due to lack of solubility of pellet in negative control 1 (Figure 4-10), here only consider negative control 2 value as reduction amount from the points in P 1000 and P 500 figures.

This suggest the method accurately work and reduction of this negative control values are done to get the degree and kinetics of aerobic biodegradation (section 4.9, 4.10, 4.14).

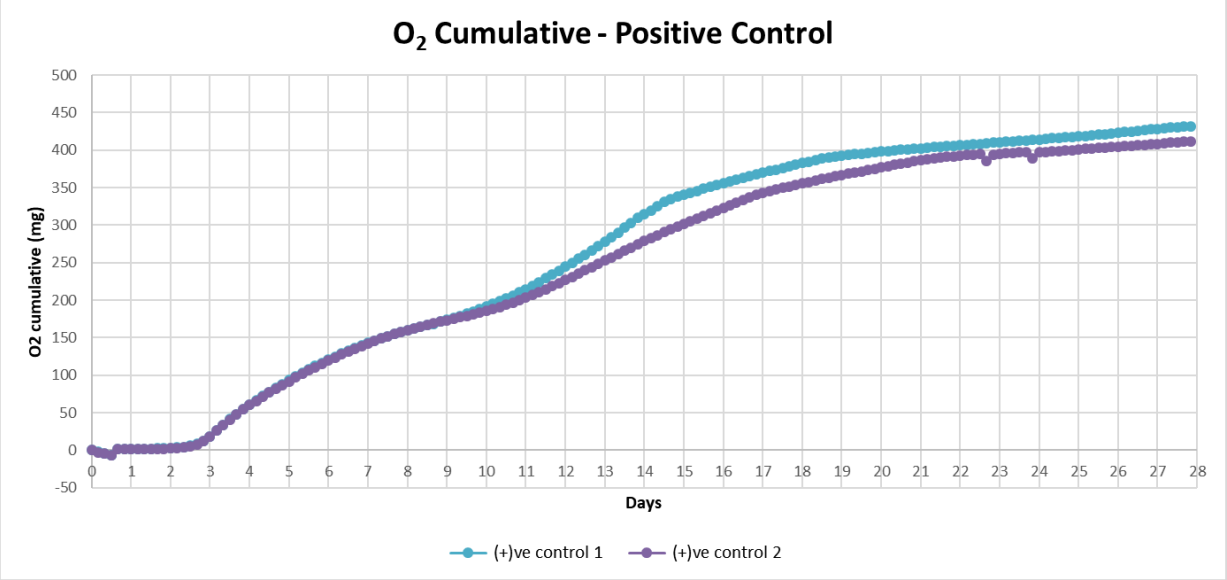

Figure 4-9: Cumulative O_2 utilization of positive controls in Micro-Oxymax test 1

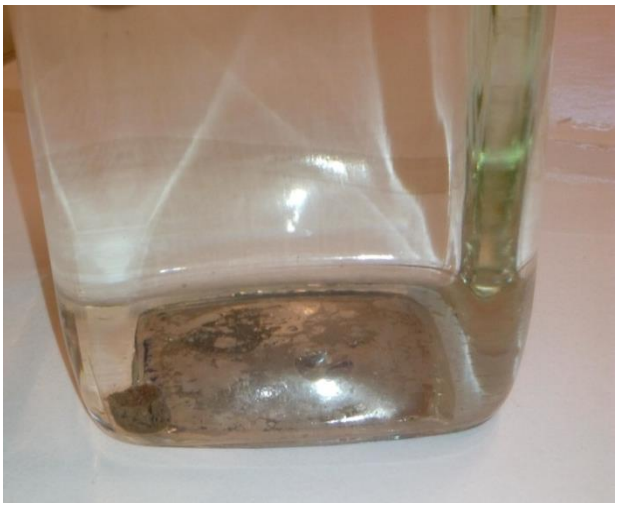

Figure 4-10: Undissolved P 500 pellet in the chamber

4.12 OxiTop results graphs

Figure 4-11 and Figure 4-12 related to P 80, P 200 shows similar pattern. This graphs also drawn reducing average two negative control values in each point to remove oxygen utilization of other biodegradable chemicals in the sea water solution with pellets. With the time after fully soaking of pellets with sea water, it starts to biodegrade as same as Micro-Oxymax. In the beginning of Figure 4-11 and Figure 4-12 shows negative oxygen utilization because after keep in incubator under 10 °C the solutions of chambers and air in head spaces need to come to 10 °C level slowly. This create pressure difference and shows low oxygen utilization at the beginning. The oxygen utilization increases in both P 200 and P 80 solutions with increasing biodegradation. Organic matter oxidizes using oxygen in the head space of the chambers and produce energy. As describe above portion of energy go for biomass production (cell synthesis). Mostly log phase of microorganisms occur in day 7 to day 14 periods Figure 4-11 using oxygen utilization rate of 10 mg/day (from slope of the graphs) and Figure 4-12 using oxygen utilization rate of 15 mg/day (from slope of the graphs). The utilization rate value between each pellet in P 200 and P 80 categories (sloes of graphs) are more or less similar. This oxygen uses to produce energy and biomass production. Oxygen consumption increase from about 70mg/ L to 150 mg /L in Figure 4-11 from day 7 to day 15. Averagely from 150 mg /L to 270 mg /L oxygen utilization increment occur from day 7 to day 15 in Figure 4-12. Around day 14 to day 16 the oxygen utilization rate (2.5 mg/day in Figure 4-12 and 1 mg/day in Figure 4-11) is lower than the day 7 to day 14 periods because of reducing organic matter content in the solution. And at this period cell death occurs. Cell synthesis, cell death is at equilibrium with energy production at this point. Then with further reduction of organic content oxygen utilization is reduced and the cell death is increase than cell synthesis. Because then microorganisms try to produce energy at least for survive. This will be appeared after day 28 in Figure 4-11 and Figure 4-12 if this test will continue. After Day 19 in there is again substantial growth due to degradation of most complex compound by microorganisms at the end after utilizing less complex compounds. P 200,4 and P 80,2 show high weigh than other pellets in each type and therefore contain high organic matter content. This the reason why P 200,4 shows clear increment in using oxygen than other pellets. Due to less weight difference in pellets in P 80 category, the gaps between lines in Figure 4-11 is lower than P 200 category in Figure 4-12. At the end in Figure 4-12 all lines are overlapping each other except P 80,4

(from day 20 to 28) due to remaining same amount of low biodegradable organic matter content with biomass. P 80,4 also shows less difference with other graphs lines due to less organic matter content difference remaining in the chambers at the end. At day 28 the oxygen utilization of P 200 averagely is about 274 mg/L and for P 80 averagely is about 157 mg/L and oxygen utilization rate maintain near to zero. After Day 24 in microorganisms are at stationary phase with cell growth keeping near to zero. Blank line goes near to zero giving oxygen utilization around 20 mg/l for background oxidation.

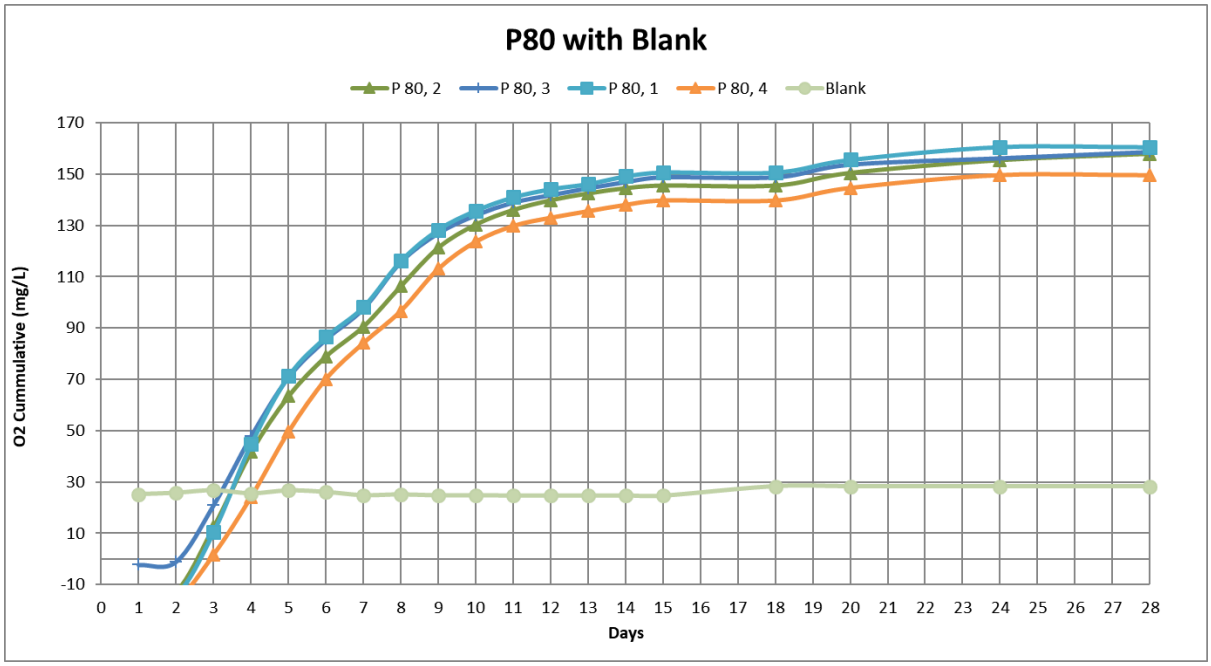

Figure 4-11: Cumulative O_2 utilization of P 80 with blank

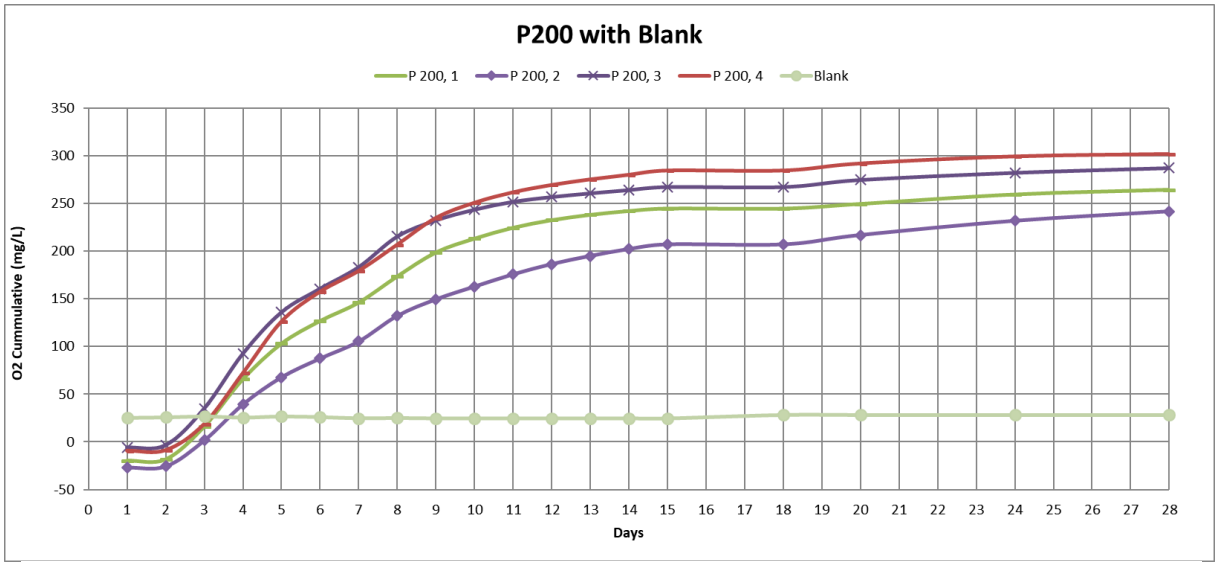

Figure 4-12: Cumulative oxygen utilization of P 200 with blank

P 200 and P 80 pellet dissolved solution contain less fat amount than P 1000 and P 500 pellet dissolved solution. Because, a noticeable fat layer is not appeared in the sea water solutions with P 200 and P 80. Therefore, oxygen utilization reduction due to fat layer is not occur in high level in OxiTop method.

Appendix F shows the positive control biodegradation. From day 7 to day 15 the oxygen utilization value changes from averagely 140 mg/L to 220 mg/L (maximum value). Both parallel overlap each other in beginning and other parts with small differences due to containing same amount of glucose in both solutions. The figure shows same pattern as P 80, and P 200. The end oxygen utilization value in positive control is about 230 mg/L. At this point most of organic matter in system is biodegraded.

Appendix F shows negative control graphs of OxiTop method showing oxygen utilization for other biodegradable chemicals in the sea water solution with pellets. The pattern of the graph lines figure for two parallels have small differences. Averagely at the end shows 45 mg/l oxygen utilization.

Deduction of negative control value need to accurately calculate kinetics and degree of aerobic degradation of fish pellets. In marine fjord system, both aerobic and anaerobic degradation can occur with available oxygen in water. Taking maximum oxygen utilization value at day 28, in Figure 4-12 and Figure 4-11 as ultimate BOD (UBOD) of system averagely for P 200 as 274 mg/L and for P 80 as 157 mg/L.

When half of the organic compound oxidation occur aerobically around day 5-7 in P 200 and P 80. Form this $t_{1/2}$ (time where 50% of the final oxygen consumption reach) can get an idea about how many days require for fully aerobically degrade of uneaten P 200 and P 80 fish pellets and parts in marine condition by bacteria.

4.13 Difference between ThOD and BOD values in P 200 and P 80 solutions and positive control

When consider about top line of each graph P 200,4 and P 80,2 in Figure 4-12 and Figure 4-11 respectively the oxygen utilization value for maximum organic matter degradation by microorganisms are 285 mg/ L and 149 mg/L. In chamber the solution volume is about 404 ml with nutrients.

Therefore,

$$Oxygen\ utilization\ of\ P\ 200{,}4\ in\ 404\ ml\ solution = \frac{285\ mg}{1000} \times 404 = 115\ mg\ O_2 \quad (4.44)$$

$$Oxygen\ utilization\ of\ P\ 80{,}2\ in\ 404\ ml\ solution = \frac{149\ mg}{1000} \times 404 = 60\ mg\ O_2 \quad (4.45)$$

ThOD value (assume same as COD) of P 200 is 140 mg O_2 and P 80 is 90 mg O_2 (Table 4-2) for average weight of two types.

The difference between ThOD and BOD value is same reasons as P 1000 and P 500 except remaining fat layer. Therefore, this difference due to low biodegradable compounds, biomass yield and remaining pellets particles in the chambers without dissolving in the sea water.

When consider positive control,

$$Oxygen\ utilization\ of\ positive\ control\ 1\ in\ 404\ ml\ solution = \frac{220\ mg}{1000} \times 404 = 88\ mg\ O_2 \quad (4.46)$$

ThOD value (assume same as COD) of positive control is about 107 mg O_2(equation (4.33)). This suggests the system gives reproducible and sensible accurate results.

When taking ThOD value as UBOD and considering the difference of it with maximum cumulative BOD value, the degree of aerobic degradation after 28 days for P 200 is about 82 % and for P 80 is about 67%.

4.14 Second experiment results of Micro-Oxymax and OxiTop

For the second experiment P 1000 and P 200 grinded pellets and autoclaved grinded pellets used with whole pellets to compare the results. Grinded pellets increase the dissolution and aerobic degradation of pellets due to increase surface area of with small particles. During autoclavation with 121 °C temperature support the hydrolysis of complex organic matter giving fully high percentage of aerobic degradation than grinded and whole pellets. Preparation of autoclaved solution and amount of autoclaved solution addition determined as in equations from (4.21) to (4.26).

Assuming high amount of ThOD as P 1000 pellet equation (4.16) fish waste sample also included into Micro-Oxymax method due to continuously refresh head space gas supplying enough oxygen for aerobic degradation. Amount of fish waste addition determine as equation (4.20)

The cumulative average cumulative graphs for whole pellet, grinded pellet and grinded autoclaved pellets for P1000 and P 200 are shown in Figure 4-13 and Figure 4-14. The figures separately for whole, grinded, and grinded autoclaved varies as figures in Appendix G and Appendix H.

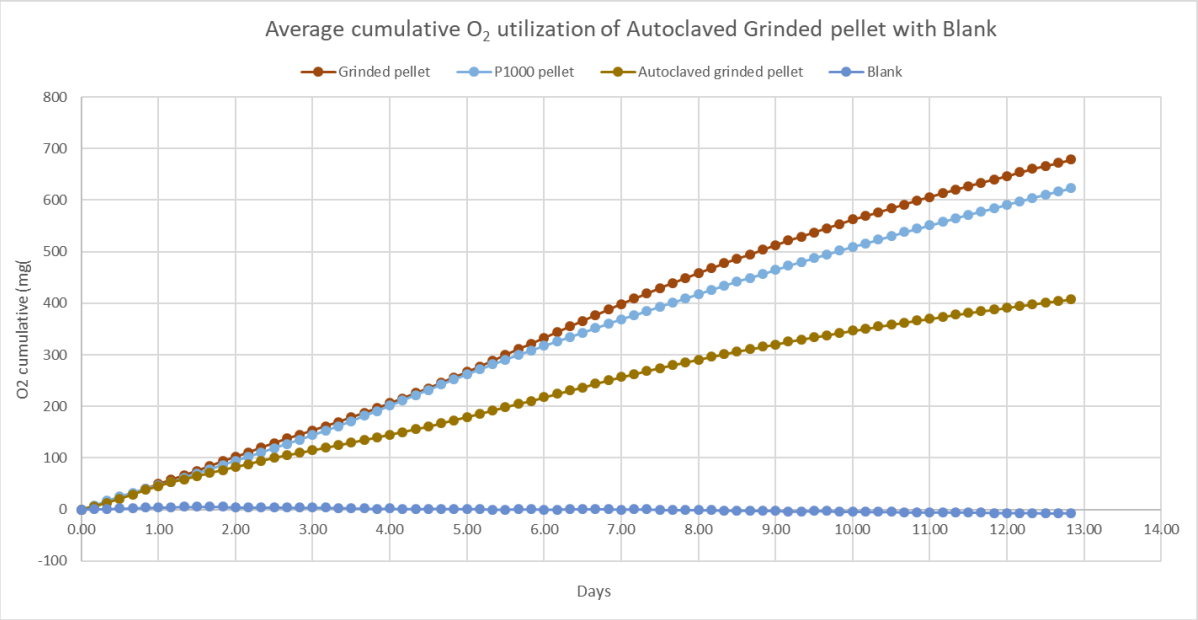

Figure 4-13: Average cumulative oxygen utilization graphs for whole pellet, grinded pellet, and grinded autoclaved pellets for P 1000 with average blanks

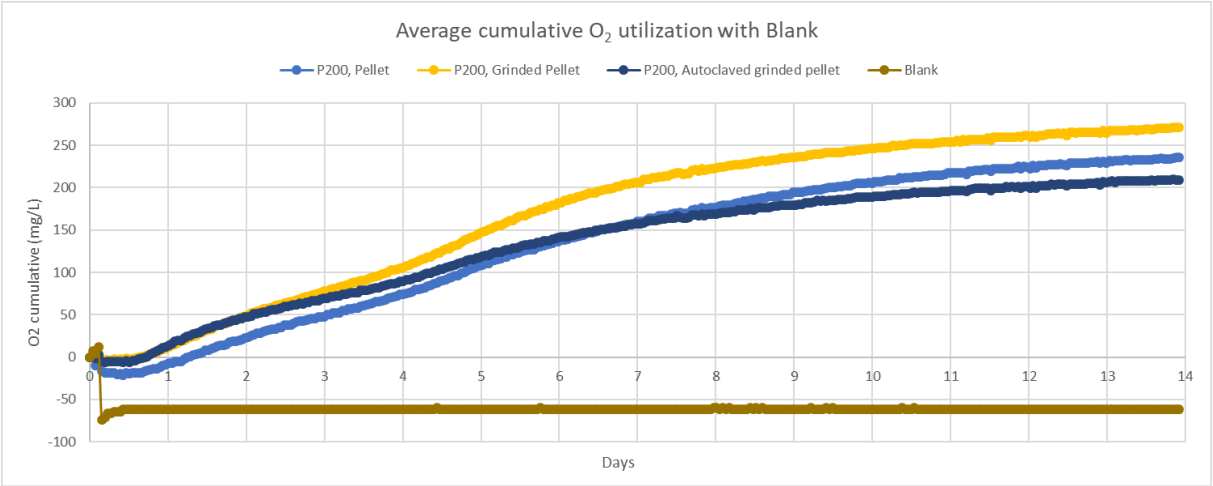

Figure 4-14: Average cumulative oxygen utilization graphs for whole pellet, grinded pellet, and grinded autoclaved pellets for P 200 with average blanks

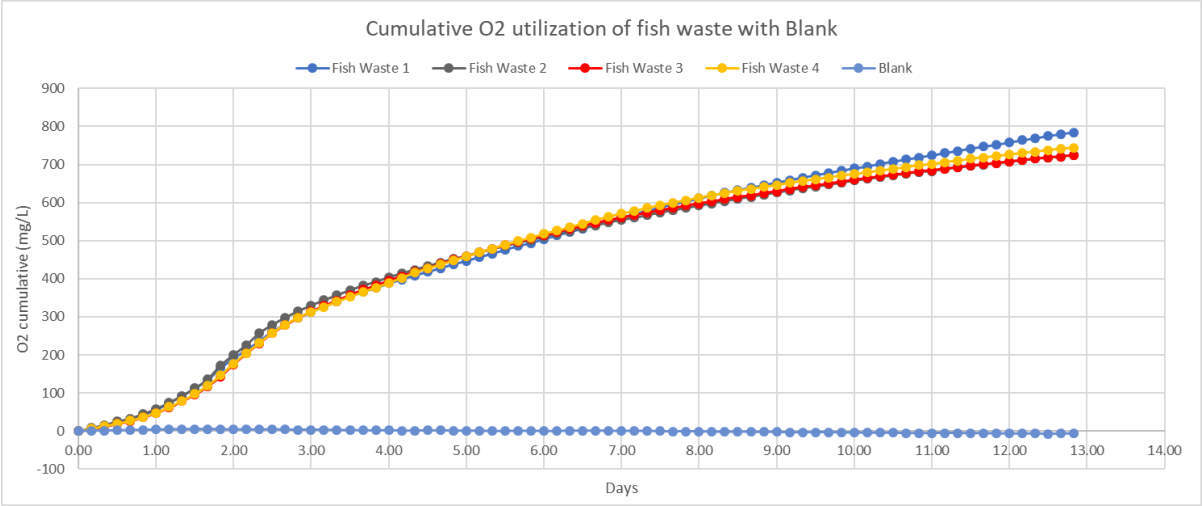

Figure 4-15: Cumulative oxygen utilization graphs for fish waste solutions

In both Figure 4-13 and Figure 4-14, when considering the values at the end, it varies as Grinded pellet> whole pellet> autoclaved grinded pellet. This happen when taking autoclaved sample for the experiment the volume may not contain the ratio of compound as pellets. But grinded pellet value compares with whole pellet the BOD values are reasonable. Because with grinding, increase the dissolution rate and the surface area to degrade for microorganisms. If autoclavation do only for one pellet and use whole volume in chamber the results will be changed and probably the autoclaved grinded sample give the maximum BOD values.

For fish waste cumulative oxygen utilization change as Figure 4-15 with time. From day 1-4, there is a higher oxygen utilization rate due to high due to usage of oxygen to produce biomass and energy by bacteria. At this movement, after using the simple compound first and then go for complex substrate utilization as protein and fat. In Figure 4-16 shows the way of oxygen percentage of fish waste containing chambers changes with time. The oxygen utilization rate

by microorganisms are higher than the oxygen supply rate to head space during that time gap. The figure lines for each parallel sample behave more or less the same way and giving maximum oxygen utilization about 750 mg/L in day 12. These figure lines are used to calculate kinetics of aerobic degradation of fish waste in marine fjord water in section 4.15 and with the COD value after BOD test, calculate degree of aerobic degradation of fish waste after 12 days.

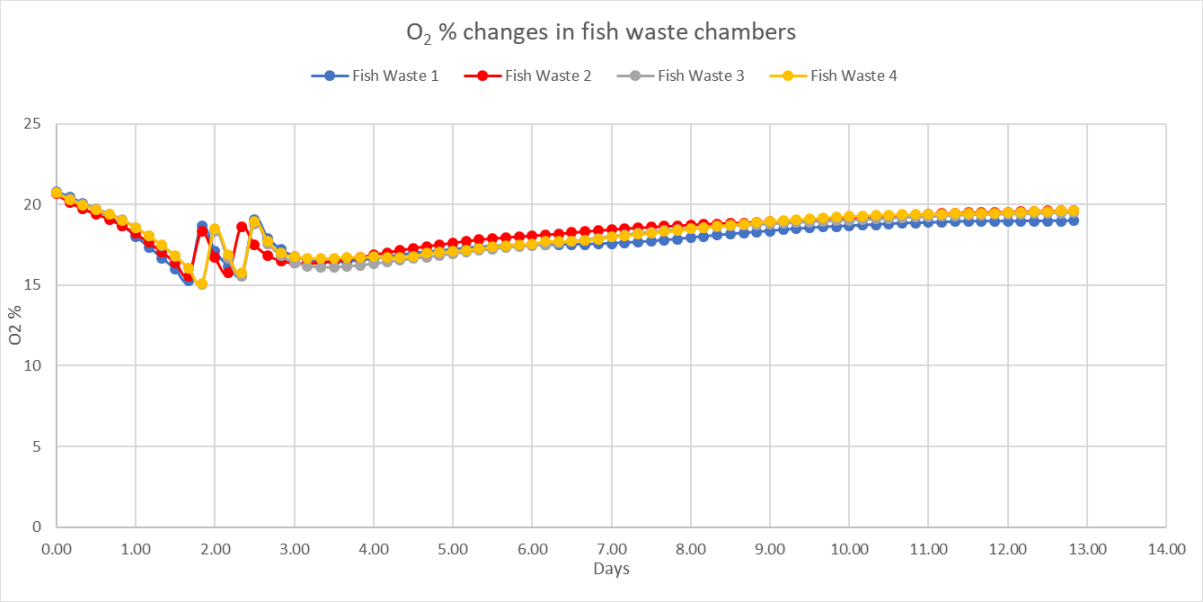

Figure 4-16: Oxygen percentage changes in fish waste containing chambers during BOD test with time

4.15 Determination of the first order reaction rate constant for aerobic biodegradation for fish pellet solutions and fish waste solutions

Cumulative graphs given in Figure 4-4, Figure 4-6, Figure 4-11, Figure 4-12, Figure 4-13, Figure 4-14, Figure 4-15 tally with the equation (4.47) (Tchobanoglous et al., 2002).

$$BOD\ (t) = UBOD\ (1 - e^{-k_1 t}) \tag{4.47}$$

$$UBOD = Total\ or\ ultimate\ carboneous\ BOD\ \left(\frac{mg}{L}\right) = (BOD_{Final} - BOD_{Initial}) \tag{4.48}$$

If this experiment continues more than 28 days, the BOD is increased little bit by aerobically degrading dead microbial cells. Here, UBOD value is considered as the difference between highest value from cumulative graphs at day 28 and the initial value (after removing the blank values). Determined k_1 values (pseudo first order degradation coefficient) for each pellet are given in Table 4-5, Table 4-6, Table 4-7.

These rate constant values give an idea about the degree of biodegradation. The highest k_1 value gives for the highest rate aerobically biodegrade sample within 28 days.

Table 4-5: Rate constant value (k_1) for each pellet type (1st experiment: 28 days)

Pellet/ Sample	k_1 value (1/d)	Average k_1 value (1/d)
P 1000,1	0.0001	0.0001
P 1000,2	0.0002	
P 1000,3	0.0001	
P 1000,4	0.0001	
P 500, 1	0.0003	0.0003
P 500, 2	0.0002	
P 500, 3	0.0002	
P 500, 4	0.0003	
P 200,1	0.09	0.09
P 200,2	0.06	
P 200,3	0.11	
P 200,4	0.10	
P 80,1	0.08	0.08
P 80,2	0.07	
P 80,3	0.09	
P 80,4	0.07	

Table 4-6: Rate constant value (k_1) for each pellet type (2nd experiment: 12-14 days)

Pellet/ Sample	$k_1$value (1/d)	Average $k_1$value (1/d)
P200, P1	0.18	0.19
P200, P2	0.19	
P200, P3	0.20	
P200, Grinded Pellet 1	0.23	0.22
P200, Grinded Pellet 2	0.22	
P200, Grinded Pellet 3	0.21	
P200, Autoclaved grinded pellet 1	0.22	0.25
P200, Autoclaved grinded pellet 2	0.29	
P200, Autoclaved grinded pellet 3	0.22	
P1000, P 1	0.13	0.14
P1000, P 2	0.14	
P1000, P 3	0.14	
P1000, Grinded Pellet 1	0.14	0.13
P1000, Grinded Pellet 2	0.13	
P1000, Grinded Pellet 3	0.13	
P1000, Autoclaved grinded pellet 1	0.16	0.15
P1000, Autoclaved grinded pellet 2	0.15	
P1000, Autoclaved grinded pellet 3	0.14	

Table 4-7: Rate constant value (k_1) for fish waste (2nd experiment: 12 days)

Pellet/ Sample	k_1 value (1/d)	Average k_1 value (1/d)
Fish Waste 1	0.18	0.19
Fish Waste 2	0.20	
Fish Waste 3	0.20	
Fish Waste 4	0.20	

In second experiment k_1 value is not for 28 days (Table 4-6 and Table 4-7). Therefore, it is not for maximum BOD value for each solution. But can get a comparison of each solution. With grinding increment of k_1 value suggest the rate of aerobic biodegradation increment due to increase the dissolution and access for bacteria to pellet particle with increasing surface area. Further increment occurs with autoclavation due to hydrolysis process of complex compounds as carbohydrate, protein and fat with heat as pretreatment for aerobic biodegradation. After the half of the 12-14 days the k_1 value for P 1000 and P 200 pellet is higher than after 28 days due to usage of organic matter is higher by bacteria for 12-14 days than considering whole process with 28 days.

k_1 vale for fish waste is same as k_1 value for P 200 whole pellet after half time completion of BOD test. Fish waste is a concentrated solution (see Appendix D) and easy to degrade by bacteria than a pellet. So, it can have higher k_1 value (Table 4-7) than P 1000 pellets due to accessibility higher for organic matter in fish waste than a whole pellet.

From both k_1 value and degree of biodegradation can get an idea about to which level the aerobic biodegradation occurs in uneaten pellets and pellet parts in fjord system.

4.16 Analysis results after BOD tests for fish pellets in 1st and 2nd experiments for fish pellets

By analyzing the solutions after the BOD test give an idea about number of other compounds as ammonia, phosphate, nitrate, total phosphorous, total nitrogen, organic matter release to the water to nutritionally enrich water causing eutrophication after biological degradation.

4.16.1 TCOD and SCOD

From TCOD value of the solutions after BOD, can calculate the TCOD value for each volume used for Micro-Oxymax (704 ml) and OxiTop (404 ml) as follows;

$$TCOD\ value\ after\ BOD\ test\ in\ P\ 1000{,}1 = 197\ mg/l \tag{4.49}$$

$$TCOD\ value\ after\ BOD\ test\ in\ P\ 1000{,}1\ in\ 704\ ml = \frac{197\ mg}{1000\ ml} \times 704\ ml = 139\ mg\ O_2 \tag{4.50}$$

Considering P 200,1, as an example for OxiTop;

$$TCOD\ value\ after\ BOD\ test\ in\ P\ 200{,}1 = 97\ mg/l \tag{4.51}$$

$$TCOD\ value\ after\ BOD\ test\ in\ P\ 200{,}1\ in\ 404\ ml = \frac{97\ mg}{1000\ ml} \times 404\ ml \quad (4.52)$$
$$= 39\ mg\ O_2$$

Like this calculate for other pellets in P 1000, P 500, and P 200 (Appendix I).

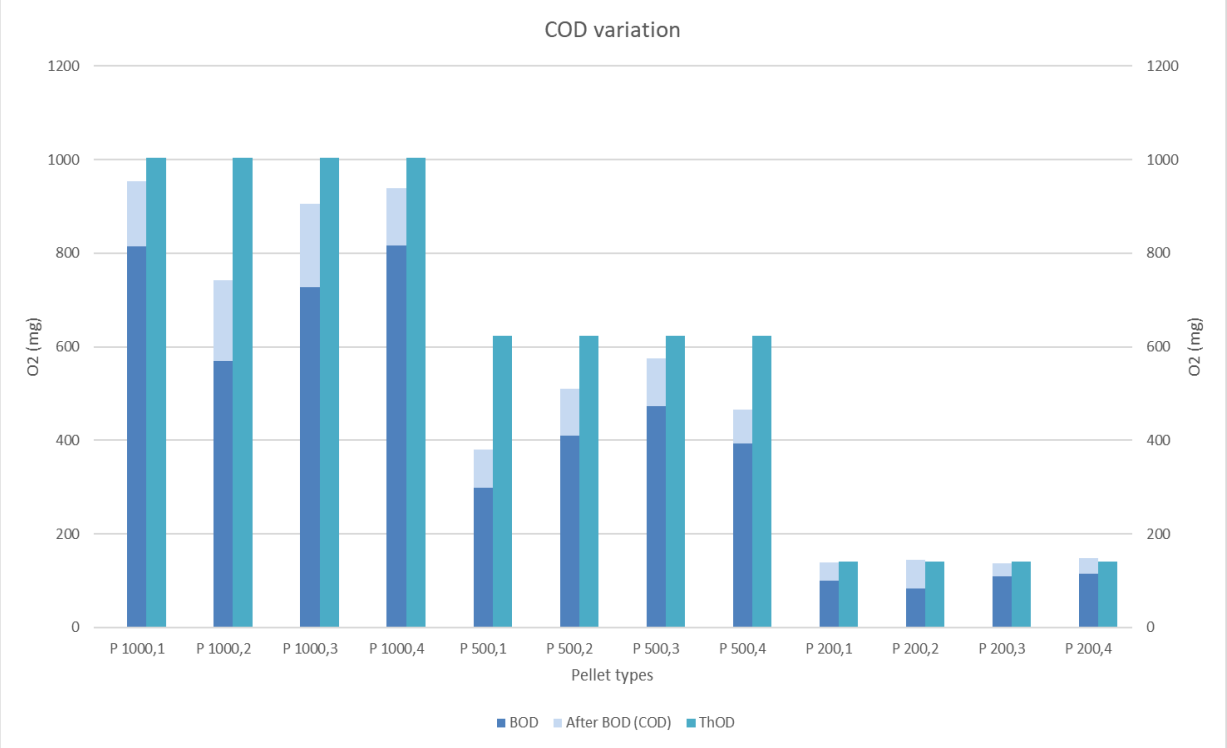

Figure 4-17: Comparison of oxygen demand before BOD with BOD and oxygen demand after BOD in 1st experiment

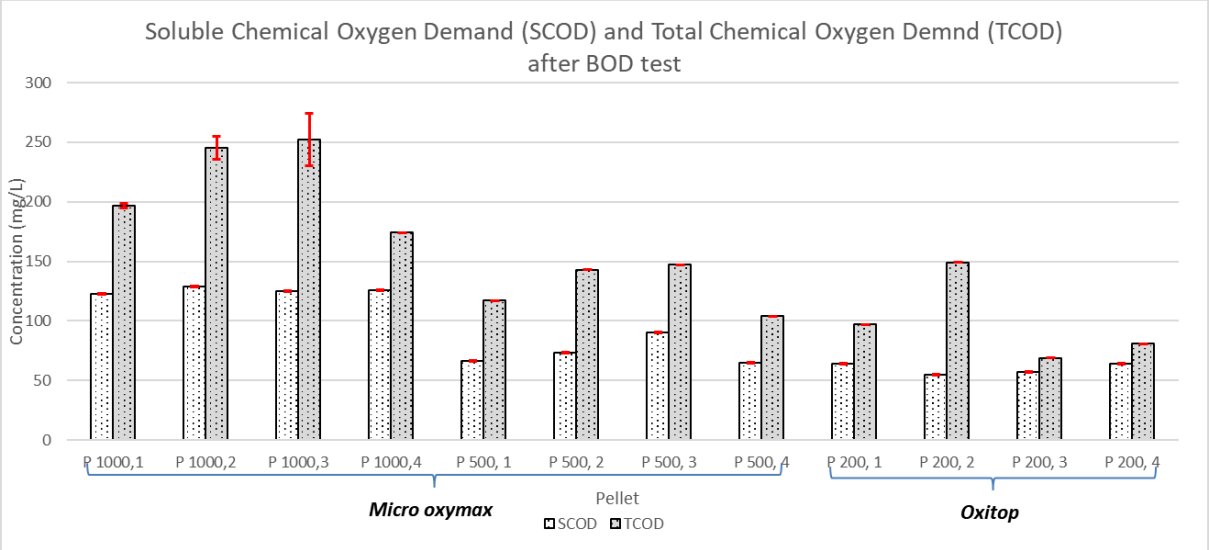

Figure 4-18: Soluble Chemical Oxygen Demand (SCOD) and Total Chemical Oxygen Demand (TCOD) after BOD test

Figure 4-17 shows the oxygen demand values which come from ThOD (consider same as COD of the pellets before BOD test), oxygen demand which come from BOD test after reducing blank and negative control effect (Micro-Oxymax and OxiTop methods) and oxygen demand of the solutions after BOD test in one figure. Here experimental result of TCOD of the fish pellet before BOD test is not consider due to low value than actual value. On behalf of that here consider ThOD value shows in one bar in Figure 4-17. Theoretically the summation of BOD value and COD in remaining solution should be more or less equal to ThOD values (initial TCOD values) in each pellet. Practically it varies as in Figure 4-17.

As explained before, BOD different in each type of pellet and why ThOD difference from BOD in each pellet (see sub chapter 4.6). The low biodegradable substances and biomass in the solution degrade chemically after BOD. After biological oxidation some complex compounds becomes easy to chemically oxidation even though such compounds can't be able to degrade by microorganisms. This may be the reason why the summation of BOD and TCOD after BOD higher than ThOD in before BOD in P 200, 2 and P 200, 4. Graphs for other pellets are showing lower value in summation of BOD value and COD after BOD value, than ThCOD value. The difference is low in P 200 pellets and high in P 1000,2 (about 261 mgO_2), in P 500,1 (about 243mgO_2), in P 500,4 (about 157 mgO_2). This is because when calculating ThOD average weight of pellet samples were taken. And the weight of pellet in each chamber in BOD test is not exactly same as the average weight. When the weight difference increase, the difference of the ThOD and summation value becomes higher. Further when calculating ThOD specific theoretical oxygen demand values were taken theoretically and TVS experimentally. Therefore, ThOD value comes from combination of experimental and theoretical values. This is not exactly same to experimentally analysis COD. Probably fully experiment value is lower than this value.

However, the remaining organic matter content is the organic matter content available for negative environmental impacts as eutrophication, if the uneaten pellets fully degrade aerobically by microorganisms present in sea water with enough oxygen.

As in sub chapter 4.6 refer to Table 4-3 describe the reason why the analysis chemical analysis results lower in fish pellet sample before BOD test. Same reason occurs after BOD test also because of fat floating on the top of the solution (especially for P 1000 and P 500 pellets containing chambers (Figure 4-7) and remaining undissolved particles in all chambers (Figure 4-8). But the reduction due to combined compounds with fat and particles are lower after BOD chambers than before BOD test solution.

The TCOD and SCOD values variation is shows in Figure 4-18 and Appendix I. SCOD represent significant amount from TCOD which means the organic content which is soluble in solution is significant. For some pellets as P 1000,1, P,200,1, P 200,4, P 200,3, P 500,3 it is more than 50% Figure 4-18. Table 4-9 also give average value about 50 – 60% SCOD in each pellet solution.

Table 4-8: Comparison of oxygen demand BOD and COD after BOD in 2nd experiment

Name of the Sample	BOD	After BOD (COD)	Sum of BOD and after BOD (COD)	Degree of biodegradation (%)
P 200 pellet	120	61	181.013	66
P 200 grinded pellet	133	197	330.12	40
P 200 grinded autoclaved pellet	109	113	221.64	49

Even though, the with remaining particles and fat layer, difficult to get COD value 100% accurately, when considering the summation of BOD value and TCOD value after BOD as UBOD value (Appendix I, Figure 4-17) , the average degree of aerobic biodegradation for P 1000 pellet is about 83%, for P 500 is about 81% and for P 200 is about 72%.

Considering same for second experiment the average degree of aerobic biodegradation for P 200 whole pellet is 66%, for P 200 grinded pellet is 40% and for P 200 autoclaved pellet is 49% (Table 4-8). For P 200 pellet it is degree of aerobic degradation is a reasonable percentage after 14 days (66%) compare with after 28 days (72%). This suggest more than half of the organic matter percentage is already biodegrade aerobically after 14 days.

Table 4-9: Summary of average chemical analysis results for each pellet category after BOD test

Pellet / Sample	TCOD (mg/L)	SCOD (mg/L)	PO_4 (mg/L)	TP (mg/L)	NH4 (mg/L)	TN (mg/L)	Ph	TS (g/L)	TFS (g/L)	VS (g/L)	TSS (g/L)	FSS (g/L)	VSS (g/L)
P 1000	217±19	128 ±1	8.29± 0	9.66± 0	26.63± 2	32.5±2	7.04 8	40±2	30 ±0	10 ±2	0.79 ±0	0.35 ±0	0.44 ±0
P 500	128 ±10	73.5± 6	6.83± 0	7.55± 1	15.88± 1	20±2	7.08 4	41 ±2	30 ±0	10 ±2	0.64± 0	0.29 ±0	0.35 ±0
P 200	99±18	60±2	4.43± 0	5.74± 0	14.13± 0	16±1	7.88 3	39 ±1	30 ±0	9±1	0.75± 0	0.43 ±0	0.31 ±0
P 80	-	-	3.7±0	4.76± 0	9.5±0	12.75± 0	8.12 8	46 ±3	30 ±0	16 ±3	0.67± 0	0.37± 0	0.30 ±0

4.16.2 Total phosphorous and Phosphate

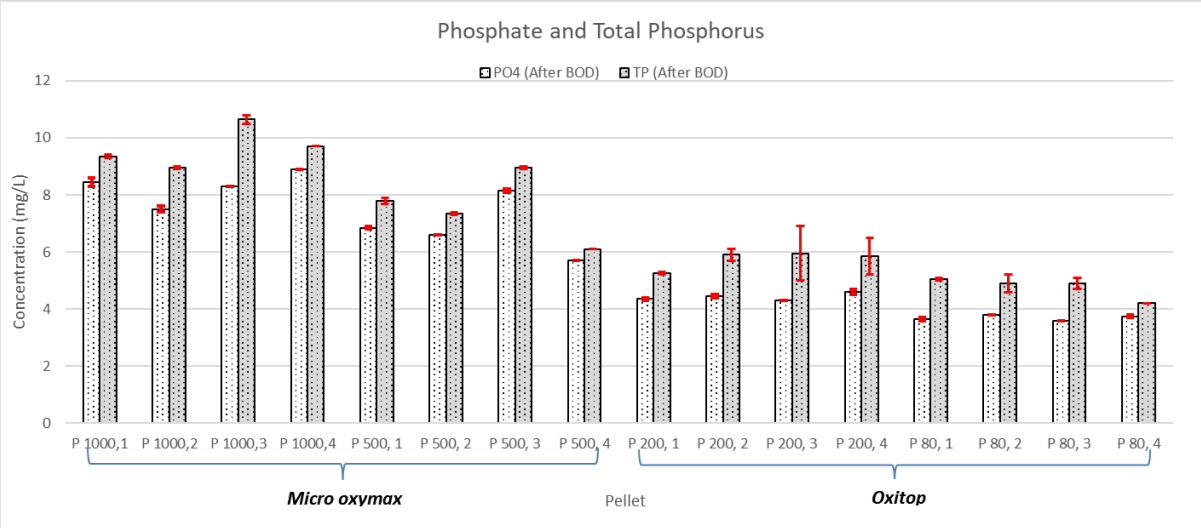

Figure 4-19: Phosphate and Total Phosphorus after BOD test

Figure 4-19 with Appendix I shows the phosphate and total phosphate amount variation after BOD test in each pellet with standard error bars. Phosphorous mainly present in nature as phosphate which is giving major part to energy storage molecule as ATP and also synthesizing of nucleic compounds in cells. According to the organic matter content due to weight difference in P 1000 pellets, P 1000,2 pellet contain less amount of organic matter and inorganic matter content. Therefore P 1000,2 give lesser amount of total phosphorous and phosphate content than other chambers in P 1000 category. Even though P 1000,4 contain highest weight highest total phosphorous content is given by P 1000,3 pellet containing

solution (10.65 mg/L). But P 1000,4 pellet containing solution gives highest phosphate content (8.9 mg/L). The reason for less amount of phosphate in P 1000, 3 than P 1000, 4 chambers is due to high fat and particle remain in P 1000,3 chamber than P 1000,4. For P 1000 category total phosphorous content is about more than 8.5 mg/L and phosphate is about more than 7 mg/L. As average value for P 1000 pellets total phosphorous is about 10mg /L and phosphate is about 8 mg/L (Table 4-9). Therefore, about 80 % of phosphorous in solution present as phosphate after BOD test for P 1000 pellets. Before BOD also the phosphate content to total phosphorous is about 80% (ratio between TP: PO_4 is 56: 46 (Table 4-3).

P 500, 3 also contain high amount of total phosphorous and phosphate content (about 8.95mg/L and 8.15 mg/L) due to highest organic and inorganic content with highest amount of weight of P 500,3 pellet. It is higher than some of P 1000 pellets due to high fat layer remaining in P 1000 pellets than P 500 pellets. As an average it is 6.83 mg/L phosphate and 7.55 mg/L total phosphorous in P 500 pellets (Table 4-9). It is about 91 % (w/w%) phosphate from total phosphorous amount.

The weight difference in P 200 pellets and P 80 pellets during each pellet in each category is low (Table 3-1). Therefore, the total phosphorous and phosphate content is more or less same in each sample. Small difference can be seen due to the percentage solubility and fat content of each pellet in chambers. P 200,2, P 200,3 and P 200,4 pellets containing chambers show about 5.9 mg/ L total phosphorous amount and P 200,1 contain 5.2 mg/L total phosphorous amount may be due to low weight than other pellets (Table 3-1). However, the Phosphate amount in four pellets in P 200 category is about t 4.43 mg/L. The average percentage of phosphate to total phosphorous is 77% for P, 200 pellets (Table 4-9).

P 80 pellets containing chamber 1,2,3 contain more or less same amount of total phosphorous content about 5 mg/L (Figure 4-19). P 80,2 contain lesser amount due to lower weight. Average phosphate amount to P 80 category is about 3.7 mg/L and as a percentage to total phosphorous 78% (W/W%) (Table 4-9). Figure 4-19 and Table 4-9 shows the total phosphorous and phosphate amount is decrease with pellet decreasing pellet size from P 1000 to P 80.

When neglecting the remaining fat and particle related phosphorous content in solutions after BOD test, the phosphorous content is significantly lower than before BOD test value due to microbial degradation. Because of phosphorous usage for energy production as ATP and biomass production in cell synthesis nuclear matter production and so on.

Normally phosphorous digestibility of fish is low in salmon varieties due to lack of enzyme (Wang et al., 2013, Kibria et al., 1997) and percentage in feed is about 1.2% (Reid et al., 2009) in Atlantic salmon fish feed. Here it lowers about 0.56% (Table 4-3). The phosphorous in organic matter convert to inorganic phosphorous as $P0_4$ mainly by microbial decomposition as heterotrophic microbes (Vanni, 2002). These inorganic phosphates take by phytoplanktons and covert again in to organic phosphorous in nutrient waste cycle in aquaculture system (see Figure 2-3). Therefore, supply nutrient for eutrophication process in farms after aerobically biodegradation of uneaten or parts of fish pellets by microorganisms (Istvánovics, 2010). Some part of phosphorous sediment in sea (Vanni, 2002).

These results from total phosphorous and phosphate content after BOD in each pellet give an idea about the phosphate comes into sea water after aerobic biodegradation. Even though the phosphate comes from one pellet is low, when consider about high number of pellets this value become high. Phosphorous is one of main nutrient for the eutrophication process (Istvánovics, 2010). Therefore, in fish farms actions must take to reduce the amount of uneaten pellets dissolution in sea water to prevent this or have to include low amount of phosphorous content when formulating fish feed.

4.16.3 Total nitrogen, nitrate, and ammonia

The total nitrogen and ammonia variation in the fish pellets containing chambers after BOD test is in Figure 4-20 and Table I-. Before BOD the ration of concentrations of TN: NH_4:NO_3 is as 4:3:1 (Table 4-3). Percentage value (w/w%) is also mentioned in Table 4-3, but the values are lower than expected. It can be clearly seen because after BOD test the total nitrogen values higher in P 1000 pellets than before value. But it should be other way round. Considering average values for TN and NH_4 for P 1000 pellets (Table 4-9) the concentration ratio is 36:27 (about 6:5). The ratio also high in after BOD chambers.

Considering Figure 4-20, all pellets give the values according to the weight of the pellets. Small variations occur due to insoluble particle amount in each chamber. Even though the organic matter content is high with weight of the particles in chambers, if the remaining insoluble matter is high the value for TN and ammonia are going down. As highest weight pellets containing chambers P 1000,4 gives total nitrogen value of 32 mg/L and ammonia value of 31 mg/L Figure 4-20. Average values are as 32.5 mg/L total nitrogen and 26.63 mg/L ammonia for P 1000 pellets (Table 4-9). Highest weight containing P 500,3 containing chamber gives highest total nitrogen as 23.5 mg/L and highest ammonia content as 18.5 mg/L. Averagely total nitrogen is 20 mg/L and ammonia is 16 mg/L in P 500 category (Table 4-9). Even though P 200,4 have little bit higher organic content than P 200,3, P 200,3 gives the highest value for total nitrogen as 17.5 mg/L and ammonia as 15 mg/L due to containing higher amount of insoluble particle remain in P 200,4 than P 200,3 containing chamber. P 80,2 containing high amount of organic matter in that category gives 13.5 mg/L total nitrogen and 10 mg/L ammonia. The average values are present in Table 4-9. But in every category in between each chamber the variation of results is low (Figure 4-20).

Important observation here is nitrate content in each chamber is <0.5 mg/L (Appendix I). The difference between values of total nitrogen and ammonia is very low in each chamber. This suggests no nitrification (see equation (2.6), (2.9) and (2.10)) occur during BOD test. Therefore, the results of BOD give only carbonaceous oxygen demand. Before BOD by nitrification inhibitor was not added to the chambers to prevent nitrification even though BOD test continues more than 5 days due to considering less nitrogen content. If n-allylthiourea (ATU) adds to inhibit nitrification this compound itself supplies ammonia to the chambers.

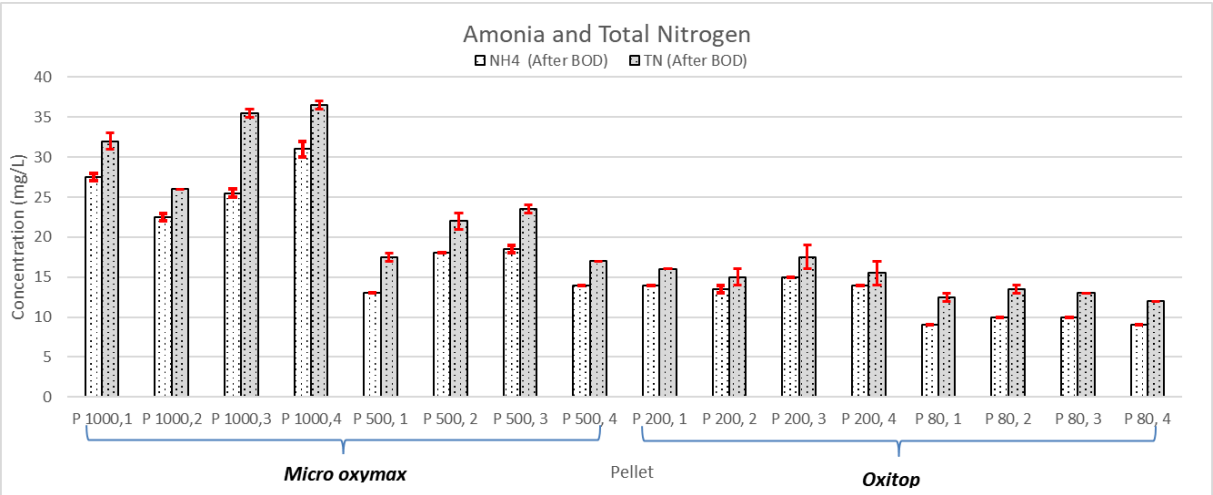

Figure 4-20: Ammonia and Total Nitrogen after BOD test

Due to protein degradation for energy production during BOD test (see equation (2.5)) ammonia come to the system and through cell synthesis process use these produce ammonias. Therefore, the ammonia is going cycling process and not retains enough ammonia for nitrification. This is because use of one pellet in chambers. In the aquaculture system lots of uneaten pellets, pellet particles and feces come into the system providing enough ammonia to nitrification (see Figure 2-1). By this BOD test give an idea about how much of ammonia come to the aquaculture system by one pellet if complete aerobic degradation occurred by microorganisms using enough oxygen. Therefore, by fully aerobic degradation reduce the amount of total nitrogen, ammonia content than content before BOD. Compare with Figure 4-19, the total nitrogen and ammonia content come to the system is higher than total phosphorous and phosphate from one pellet aerobic degradation. As example according to Table 4-9 the concentration ratio of TN: TP is about 3:1 and ratio between NH_4:PO_4 is also 3:1 for P 1000 pellet. For P 200 and P 80 also gives same ratios. For P 500 also same TN:TP ration and NH_4:PO_4 ratio is about 2:1. When consider eutrophication process both nitrogen and phosphorous removals have to consider. This ratio suggest the Nitrogen removal is higher than phosphorous removal by third from an aerobically degrade pellet.

4.16.4 TS, TFS and VS

Figure 4-21 and Appendix I shows the TS and VS variation in each chamber after BOD. VS amount shows as the percentage of total solid, means the remaining organic matter amount after BOD. This amount is the organic matter amount which can't be biodegradable by microorganisms aerobically to produce energy for survive and cell synthesis. Some compound which can't easily biodegrade by microorganisms and degrade chemically. The VS content in Figure 4-21 and TCOD content in Figure 4-18 give an idea about this. In Figure 4-21 the highest VS amount shows in P 1000,3 and in Figure 4-18 higher TCOD amount given by P 1000,3 due to both VS and TCOD represent mostly the organic content in the solution.

But the VS not present only organic matter decomposition, it contains decomposition of some minerals as well.

Total fixed solid value is more or less same for each chamber is about 30 g/L (Figure 4-21). As other results the TS and VS content is not have clear differences with weight of each pellets in each category. Normally the value should be increased with the weight increment in each category. The reason for this is the amount of particle contain in the taken portion of solution for testing is varying. Because, in every chamber particles remaining after BOD test. P80,3pellet have highest TS (56g/L), may be due to high amount of particle matter contain in the portion of solution takes for measuring. Other than P 80,3 other chambers have TS amount less than 50g/L and VS less than 20 g/L (Figure 4-21).

The variation for TCOD and VS amounts during pellets is also because of the amount of fat and particles percentage in portion which is taken for COD and VS analysis (Figure 4-21 and Figure 4-18).

The average values are given by Table 4-9. It shows the results for pellets in every category are more or less same for TS, VS and TFS. Big difference occurs in P 80 category due to P 80,3.

The TS content before BOD is about 92 % (w/w%) and from that 86% VS (organic matter) in pellets (Table 4-3). After BOD in P 1000,1 pellet it is about 37 g/L and 7 g/L VS by one pellet degradation in sea water. The TS and VS amount in chamber volume can calculated as equation (4.53), (4.54) and (4.55) (see Table I-). This amount come from about 0.6 g pellet degradation (see Table 3-3).

$$Volume\ of\ P\ 1000\ chamber = 704\ mL \tag{4.53}$$

$$The\ total\ solid\ in\ 704\ mL = \frac{37g}{1000} \times 704 = 26\ g \tag{4.54}$$

$$Volatile\ solid\ in\ 704\ mL = \frac{7g}{1000} \times 704 = 5\ g \tag{4.55}$$

This total solid amount shows the amount of suspended and dissolved solids in the solution. Total solid contain remaining particles, biomass (living and death cells), organic and inorganic substances (calcium, chloride, nitrate, phosphorous, iron, sulfur and so on) in the solution. Sea water itself contains total solid content. According to Figure 4-22 the amount of TSS is very low for each pellet. This means dissolved solid content is high in solutions. The volatile solid content (organic matter content) is less than 50% for all pellets in relation to TS content in each solution.

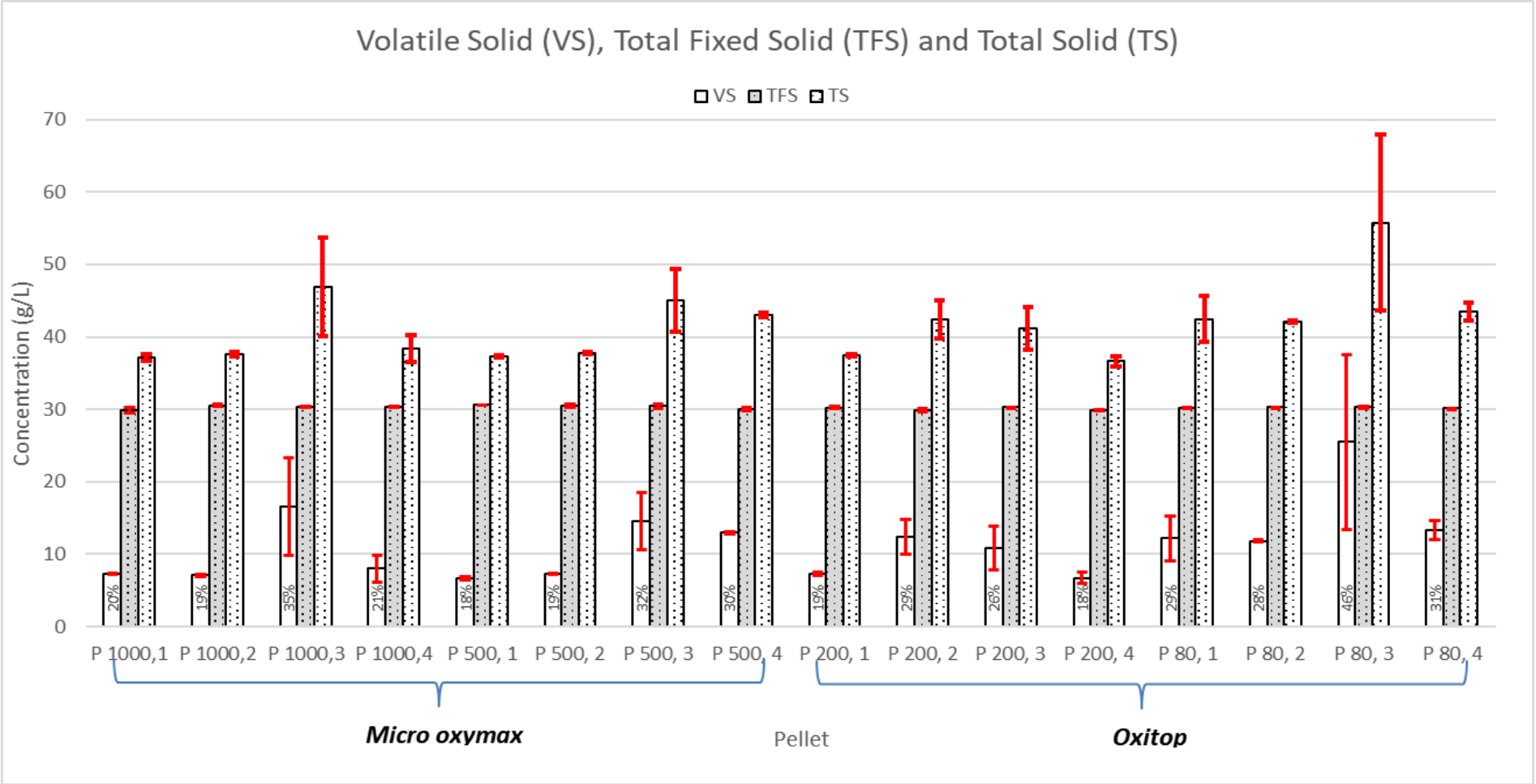

Figure 4-21: Volatile Solid (VS), Total Fixed Solid (TFS) and Total Solid (TS) after BOD test

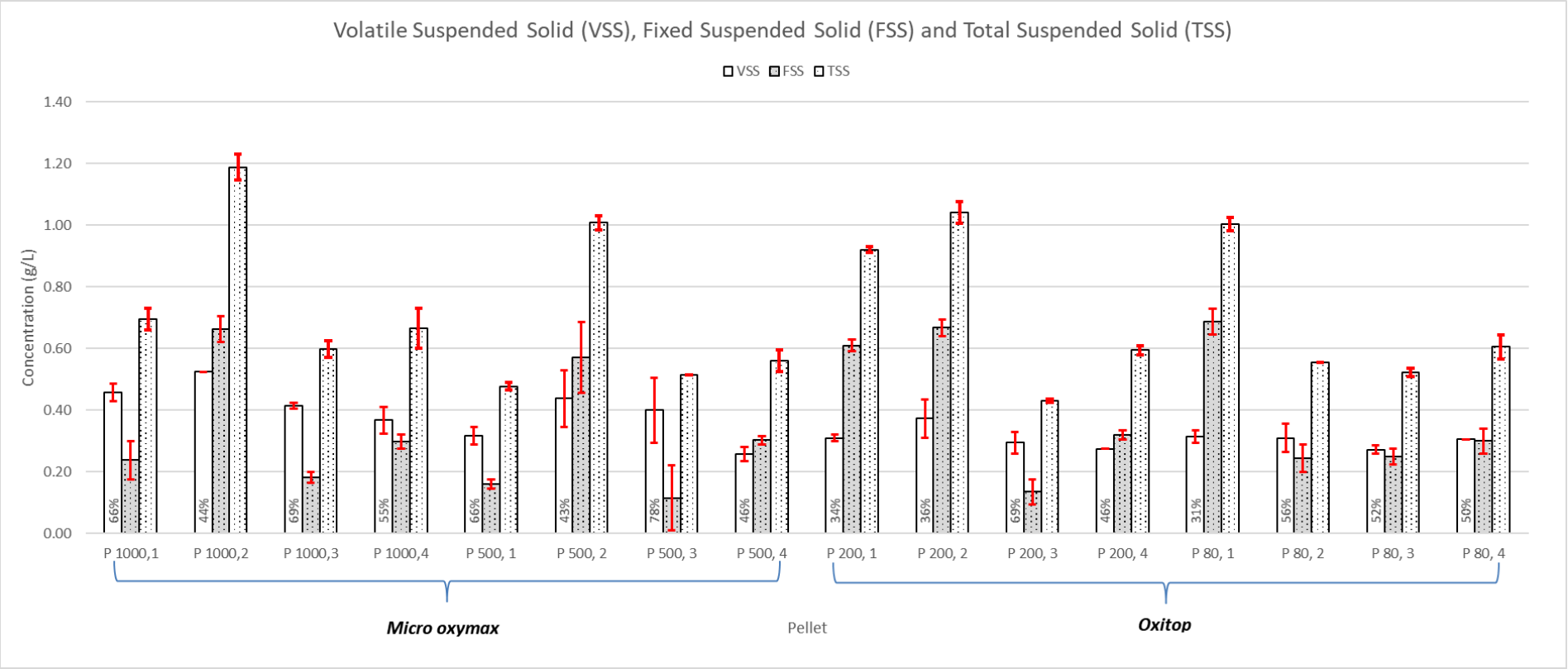

Figure 4-22: Volatile Suspended Solid (VSS), Fixed Suspended Solid (FSS) and Total Suspended Solid (TSS) after BOD test

4.16.5 TSS, FSS and VSS

Figure 4-22 and Appendix I show the TSS, FSS and VSS variation in each pellet.

TSS suspended solid particle content of the solution which can't go through 1.6 µm pore size (Whatman® Glass microfiber filters, Grade GF/C filter paper). Lot of inorganic substances pass through the filter paper. This is the reason for the low TSS amount. This means ion percent in sea water do significant effect in high TS content in Figure 4-21.

But this also depends on the solid content (particles and fat) present in the solution portion takes for analysis. From VS and VSS, can get clear idea about the remaining organic compound which is dissolved in solution and with particles without dissolving.VSS amount (Figure 4-18) is very low compare with VS amount (Figure 4-21). This means more organic and also some ignited inorganic soluble in solution is higher than in suspended solid particles in solution. Means the remaining particle amount is low and this is noticeable.

When consider, Figure 4-22, P 1000,1 gives 0.7 g/L and 0.5 g/L values for TSS and VSS.

$$The\ total\ suspended\ solid\ in\ 704\ mL = \frac{0.7g}{1000} \times 704 = 0.5\ g \tag{4.56}$$

$$Volatile\ suspended\ solid\ in\ 704\ mL = \frac{0.45g}{1000} \times 704 = 0.3\ g \tag{4.57}$$

The ratio between TSS and VSS for P 1000,2 pellet is higher than other pellets (Figure 4-22). This means the organic matter content in suspended solid particle is high. This contains bacterial biomass and remaining organic matter which is not biodegrade. From VSS and VS can get an idea about the biological concentration of the solution. This is more or less same to another pellet as well (Figure 4-22).

For P 1000,1;

$$Volatile\ matter\ content\ in\ suspended\ solid\ from\ all\ volatile\ solid = \frac{0.3g}{5\ g} \times 100 = 6\% \tag{4.58}$$

This is varying from pellet to pellet. But the percentage amount is about 10% or less than 10%. This means volatile suspended matter content is less in volatile solid in each pellet.

From the difference between VS and VSS, as example for P 1000,1 4.8 g amount is responsible for SCOD value $87 mgO_2$ (Figure 4-18). Therefore, from 1g of organic matter content $18 mgO_2$ oxygen demand is given approximately. Considering This is only for P 1000,1 pellet.

$$SCOD\ represent\ forganic\ matter\ differnce\ from\ VS\ and\ VSS\ in\ P\ 1000{,}1 = \frac{123}{1000} \times 704 = 87\ mgO_2 \tag{4.59}$$

In the P 1000,1 pellet 5 g (VS) of organic matter related to 139 mgO_2TCOD amount (see equation (4.55)). Therefore, approximately from 1g of organic matter (both suspended and soluble), $27 mgO_2$amount is needed to degrade. Therefore, to degrade the organic matter content not soluble in sea water solution need 9 mgO_2 which is related to VSS.

This is an approximate value, because of the fat and particle amount in both COD and TS, VS and TSS, VSS analysis solution the value can be varying. However, compare with initial ThOD

for pellet before BOD test (see Table 4-3) this needed oxygen amount to degrade remaining organic matter content is very low. Appendix I shows above calculations for all four chambers in P 1000, P 500 and P 200 taking account the relationship between TCOD, SCOD, VSS, VS of the solutions and give an idea about remaining organic content in pellet solution after BOD.

Further organic matter which soluble in the solution is high in each chamber for all pellets and difference between VS and VSS prove it again with SCOD (see Figure 4-21 and Figure 4-22).

4.17 Analysis results after BOD tests for fish waste solutions

Table 4-10 shows the average chemical analysis results for fish wastes sample and Appendix J shows the chemical analysis results for each sample separately. This analysis results give an idea about how much TP, TN, NO_3, NH_4, PO_4, organic and inorganic matters come to the marine fjord system after partially aerobic degradation of fish waste by bacteria to support negative environmental impacts as eutrophication.

Table 4-10: Summary of average chemical analysis results for fish waste samples after BOD test

Sample	TCOD (mg/L)	SCOD (mg/L)	PO_4 (mg/L)	TP (mg/L)	NH_4 (mg/L)	NO_3 (mg/L)	TN (mg/L)	TS (g/L)	TFS (g/L)	VS (g/L)	TSS (g/L)	FSS (g/L)	VSS (g/L)
Fish waste	1455 ± 35	468 ± 42	42 ± 4	45 ± 3	28 ± 2	4 ± 0	73 ± 6	33 ± 0	28 ± 0	5 ± 0	3 ± 0	2 ± 0	2 ± 0

The ratio of TN:TP is about 3:2 after partially completion of BOD test and before BOD test (Table 4-4) shows this ratio is about 2:1. When compare with ammonia amount, nitrate amount is very little in solutions. NH_4:PO_4 is about 2:3 after BOD test and it is 1:3 before BOD test.

Comparing maximum BOD values for each solution and the TCOD values after BOD test, the total is approximately same for each sample. As example according to Figure 4-15, the lowest maximum value shows by fish waste 3 and highest maximum value by fish waste 1. TCOD value after BOD the highest value shows by fish waste 3 and lowest by fish waste 1 (Appendix J).

Taking into account summation of average difference of maximum BOD value and initial BOD value (Figure 4-15) and remaining average TCOD value in fish waste solutions as UBOD the degree of aerobic biodegradation after 12 days is about 34 %.

4.18 Settling velocity and disaggregation of pellets in shaking table

The settling velocity of each pellets variation for 1m depth with time shows in Figure 4-23 and 10 cm depth shows in Figure 4-24 (see Appendix K). At the beginning the velocity of the pellets vary as P 1000>P 200>P 500, P80. In the tube 25 m from bottom was not included into 1 m distance to avoid shear stress from the bottom and also here assume drag effect from side wall is negligible amount (Chen et al., 1999).

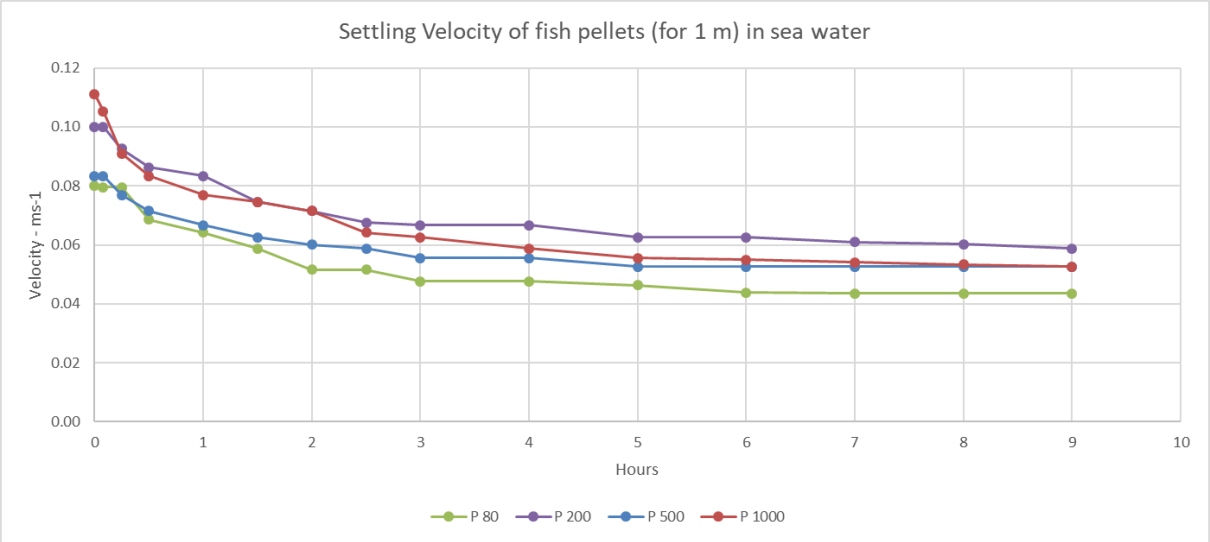

Figure 4-23: Settling Velocity of fish pellets for 1 m depth in sea water

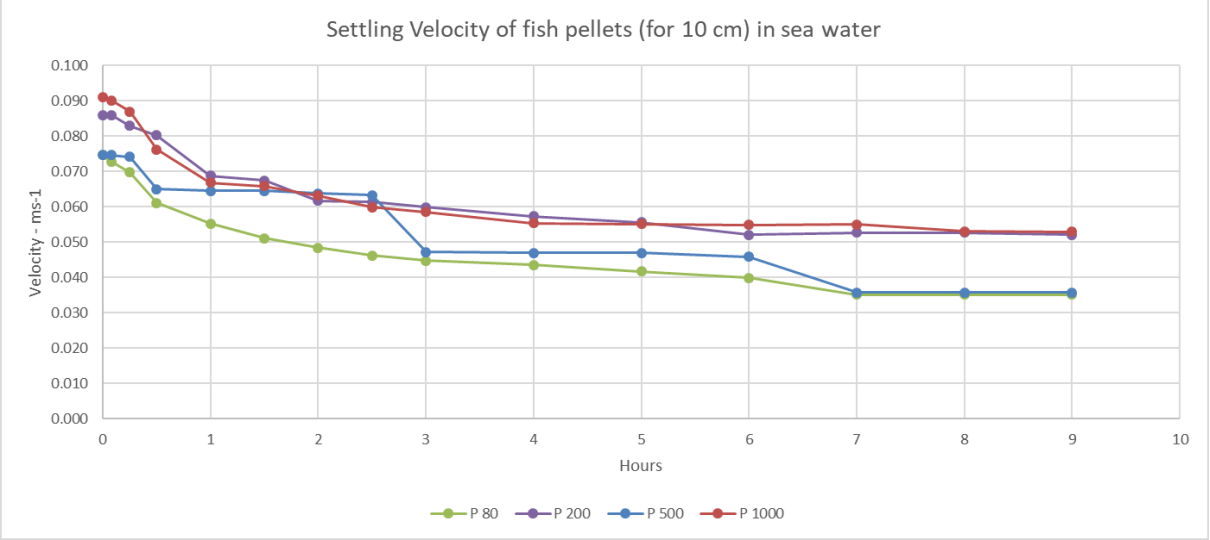

Figure 4-24: Settling Velocity of fish pellets for 1 cm depth in sea water

For 10 cm distance velocity (ms^{-1}) gives as 0.091>0.086>0.075, 0.075 respectively to each pellet and for 1m distance velocity (ms_{-1}) gives as 0.11>0.10>0.08, 0.08 with respect to each pellet at the beginning (0 hours). From these values shows, the pellets move higher velocity in first 10 cm distance.

Table 4-11: Physical characteristics of each fish pellet

Particle type	Length (mm)	Diameter (mm)	Weight (g)	Volume (mm^3)	Density (g/mm^3)	Density (kg/m^3)	Color
P 1000,1	8.86	9.25	0.639	615.54	0.001	1038.11	Light brown
P 1000,2	9.26	9.15					
P 1000,3	8.66	9.34					
P 1000,4	9.44	9.47					
Average	**9.06**	**9.30**					
P 500,1	6.26	7.39	0.345	312.54	0.001	1103.86	Dark brown
P 500,2	7.54	7.68					
P 500,3	7.00	7.62					
P 500,4	7.29	7.42					
Average	**7.02**	**7.53**					
P 200,1	5.28	5.05	0.096	107.72	0.001	891.17	Dark brown
P 200,2	4.85	5.22					
P 200,3	5.21	5.13					
P 200,4	5.41	5.17					
Average	**5.19**	**5.14**					
P 80, 1	4.31	4.40	0.053	72.94	0.001	726.66	Dark brown
P 80, 2	4.40	4.58					
P 80, 3	3.99	4.78					
P 80, 4	4.34	4.93					
Average	**4.26**	**4.67**					

According to the Table 4-11 the calculated density of each pellet varies as P 500>P1000>P200>P80. This is same for weights as well. Therefore, normally the settling velocity of pellets should be varying as P1000>P500>P200>P80. But here Figure 4-25 shows P 1000, P 500 pellets mostly cylindrical shape and P 200, P 80 pellets are round shape. From length and diameter values in Table 4-11 also suggest the round shape of P 200 and P 80 pellets. (Chen et al., 1999) also suggest the settling velocity of the pellets vary with the shape of the pellet.

Figure 4-25: The variation of shape of the pellets

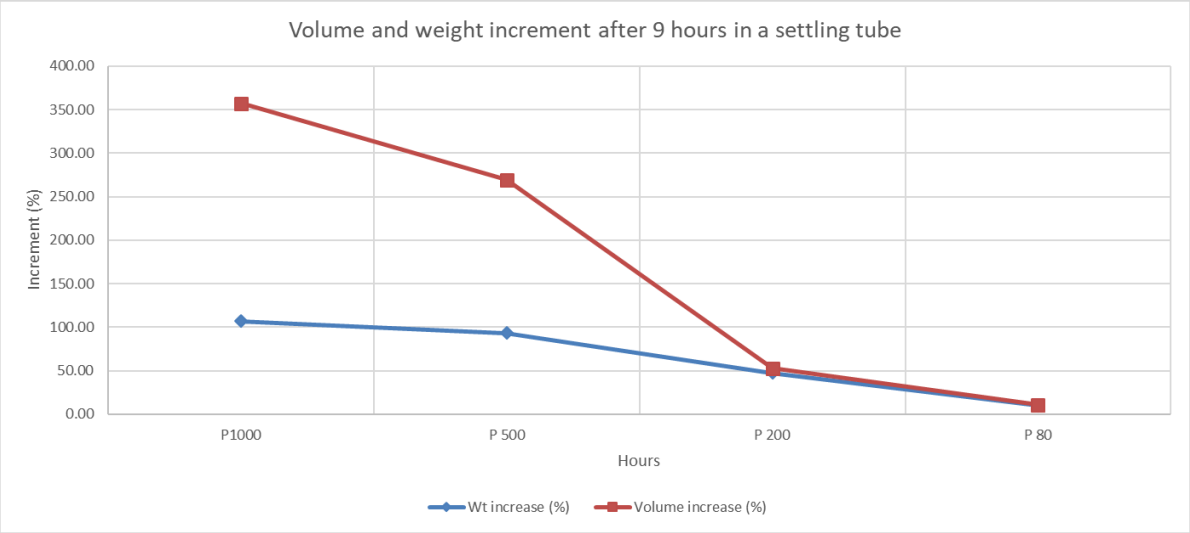

Figure 4-26: Volume and weight increment after 9 hours in a settling tube

After soaking the P 200 and P 80 pellets with sea water become more round shape with absorbing sea water. Further the degradation of pellets after soaking with sea water is higher in P 1000 and P 500. They started to degrade quickly in sea water (see Appendix L). P 1000 and P 500 pellets have noticeable fat layer on the top of water in settling tube (Figure 4-27) but negligible noticeable fat amount present in P 200 and P 80 than P 1000 and P 500. Because of the fat bubbles tend to go up, the velocity is reduced in P 1000 and P 500. Therefore, the shape, quick degradation, and fat content all together is reason for giving lower velocity for P 500 pellet than P 200. In P 1000 pellet also the velocity will increase than this if remove the effect of shape, fat amount (Figure 4-27) and quick disaggregation. Normally fat is spray to the surface of pellets to reduce friability and friction of pellets to reduce disaggregation while transport and immersion in water. But instead of fat can use attractant compound to keep the pellet particle attach together. By removing quick disaggregation can supply high nutrient amount with pellet to fish by reducing nutrient leaching happen with pellet disaggregation (Yandi and Kurtoğlu, 2016).

Appendix K describes the size and physical appearance changes of pellets after 9 hours. Figure 4-26 shows the weight and volume increment of the pellets after 9 hours. After soaking for 9 hours weight increment for P 1000 pellet -106.81 %, P 500 pellet-93.03%, P 200 pellet-47.47% and P 80 pellet – 9.84%. Size increment after 9 hours is P 1000 pellet- %, P 500 pellet- %, P 200 pellet- %, P 80 pellet - %. With the size, weight increment, organic matter leaching and the particle detachment, the density of pellets decreases and therefore settling velocity reduce in each pellet type. Further with size increment develop high resistance to fall and high friction (Piedecausa et al., 2009). After 6 hours the velocity of the pellets for 10 cm becomes constant and velocity for 1m also becoming constant. The velocity of each pellet reduces with time for every pellet due to degradation of pellet. The degree of degradation is varying from pellet to pellet. Special thing is even though pellets already degrade in to particle, pellet is not separate into parts, the particles always agglomerate and stick together, and it appears like a pellet. Therefore, the settling velocity not changes lot with time even though pellet is already degraded into particles. This agglomerated particles called as “flocs” (Law et al., 2014). In the aquaculture ponds with these flocs contaminated particles combined and go to nearby natural water system with water waves and make negative impacts.

Table 4-12: Floating time of each pellet before soaking

Particle	Floating time (s)
P 1000	35
P 500	11
P 200	2.6
P 80	1.3

Table 4-12shows the floating time of each pellet before sinking. The floating time reduce as P1000>P500>P200>P80. This is because variation of volume variation. P 1000 have higher volume than others (Table 4-11) and take more time to soak fully with sea water. Therefore, have highest floating time for P 1000 and due to smallest volume P 80 have lesser floating time. P 1000 and P 500 pellets are more extruded pellets than P 200 and P 80. Therefore, until the air bubbles inside the pellets fill with sea water the pellets tend to float. But in aquaculture farming system with the supplement of feed due to movement of fishes and the water the pellets sink quickly without floating (Piedecausa et al., 2009). Therefore, relationship between floating time and settling velocity will not applicable for real fish farming systems and further studies needed to prove it.

After settling to the bottom in cages, the fate of the pellet is described in Appendix L.

During disaggregation process of feed pellets in shaking table one pellet from P 500 (P 500, 2) act differently and not disaggregate as other pellets in same category. Possible reason for this is the changing of the ingredients amount in the pellet than others. (Yandi and Kurtoğlu, 2016) also shows that there can be a difference in pellets composition due to measurement errors in same batches.

P 1000 and P 500 pellets to keep the shape of the pellet while transporting and in the sea water the oil spray to the surface to reduce friction. But they degraded quickly than P 200 and P 80.

P 80 and P 200 pellets are harder than P 1000 and P 500 pellets because of small size. According to the degradation pattern of these pellets, can agree with the idea of hardness of pellet is increase when the size of the pellet decreases. But storage condition, processing condition, ingredients affect for the hardness of the pellet (Chen et al., 1999). Generally hard pellets have high resistance to degrade and also difficult to digest by fish. When compare with less hard pellet the loss is low in hard pellets (Yandi and Kurtoğlu, 2016). But some studies shows the feed intake of harder pellets is about 20% lower than less harder pellets (Aas et al., 2011).

Therefore, pellets hardness should be in ideal level. Because of it is too hard and too soft both loss lots of pellet without eating by fish. Somehow by increasing hardness the disaggregation can be reduced and nutrient leaching can be reduced.

In real scenario the settling velocity is lower than this due to other particles present in the sea water. Therefore, mentioned as (Hempel, 2012) this value misleading us little bit. But this experiment gives an idea about the settling velocity of the uneaten pellets in aquaculture ponds. This says time takes by pellets to settle in the bottom. From this can get an idea about the suitable time to clear the bottom in cage fish farms using water current or changing water. Furthermore the Figure 4-23 and Appendix suggest the pellet degradation time during the settling of the pellets and after settling to the bottom and effect of fat in aquaculture system using high fat containing pellets as P 1000 and P 500. Fish use to eat the pellets without degrade into particles. Therefore lots of P 1000 and P 500 pellets remain as uneaten due to the high

degradation into particles (Figure 4-28, Appendix L) cause negative environmental effect enrich nutrient content in water. This can be avoided by using eatable coating layer around the particle, using attractants without using high amount of fat spray over the pellet.

The pellet dissolution rate, degradation while settling the pellets, physical characteristics of each pellets as hardness of pellets affect for the degree and kinetics of aerobic degradation of each pellet in marine condition by degrade pellet physically into particles. Further time consume for aerobic biodegradation is less, if the pellet dissolution and disaggregation high in marine fjord system due to easily access of microorganisms to degrade pellets particles than whole pellet. Even though P 1000 and P 500 pellet disaggregate easily, P 200 and P 80 pellet contain less organic matter amount and higher dissolution rate due to less fat amount. Therefore, the rate constant of smaller pellet is higher than big pellets.

Figure 4-27: Fat bubbles floating on the top of tube for P 1000 and P 500

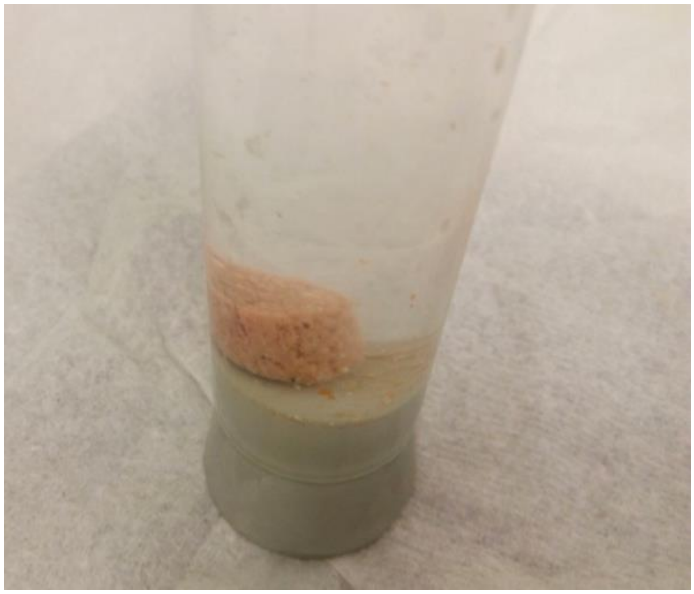

Figure 4-28: Degraded particles for P 1000

4.19 Future suggestions

Even though the respirometry method used in this study is accurate enough, BOD analysis can be done using more novel, more accurate, less time-consuming method as mentioned in section 2.8.1 to get more accurate results. The comparison of k_1 value can be done at different temperature level to develop a better model to determine the level and degree of biodegradation in marine fjord system. Due to difference composition of fish feeds, to get relationship between fish pellet and fish waste containing mainly feces, same site can be used for the experiment to collect samples of fish pellet and wastes. Without taking a volume amount from autoclavation sample, if can use whole sample volume for BOD test will give accurate results for pellets due to difficult to get the representative volume with fat bubbles. To determine the k_1 value for fish waste more accurately it is better to continue the BOD test for 28 days. Then it can compare with the k_1 values of fish feeds after 28 days BOD test.

Further using these k_1 values, settling velocity, degree of disaggregation and dissolution rate, floating time after further doing more experiments can use for developing a more accurate model for get level of aerobic biodegradation of fish pellets and fish waste containing feces by bacteria, and degree of contribution by uneaten fish pellets, fish waste as feces for the negative environmental impact in fjord system as eutrophication, saprobiation and hypoxia conditions.

5 Conclusion

- Due to formation of fat layer when dissolving P 1000 and P 500 pellet and difficulty to take compounds bound with fat layer in to solution, it is difficult to get 100% accurate representative sample before and after BOD test. However, chemical analysis results before and after BOD shows about 80 % of phosphorous present as phosphate. <0.5 mg/L of present nitrate amount after BOD test suggest giving only carboneous BOD without nitrogenous BOD. Nitrogen removal in marine water is higher by aerobic biodegradation of pellets than phosphorous (TN:TP and NH_4^+:PO_4^{3-} ratios are about 3 :1 in pellet solution after BOD test except P 500. P500 it is about 2:1). Organic matter content (VS) is less than 50% compare of TS after BOD test for pellets. These values give an idea about the amount of organic matters, ratios of inorganic matters come to the marine system before and after aerobic degradation of fish feeds which are going for nutrient cycles.

- NH_4^+:PO_4^{3-} is about 2:3 after BOD test and it is 1:3 before BOD test for fish waste suggesting more phosphate release with fish waste before and after aerobic biodegradation of fish water in marine condition. But TN:TP ratio is 3:2 after BOD and 2:1 before BOD test and this is similar to these ratios in fish pellets. These values give an idea about the amount of organic matters, ratios of inorganic matters come to the marine system before and after aerobic degradation of fish waste which are going for nutrient cycles.

- In the real scenario the full aerobic biodegradation will not occur. Part of organic matter can biodegrade anaerobically if enough oxygen not available for aerobic degradation. Considering half of the aerobic degradation by marine microorganisms, according to cumulative oxygen demand figures the time where 50% of the final oxygen consumption reach ($t_{1/2}$) after 11-13 days in P 1000, after 9-12 days in P 500, after 5-7 days in P 200 and P 80. Further from this can predict the level of completion of aerobic degradation after 28 days.

- When taking ThOD values as UBOD and considering the difference of it with maximum cumulative BOD values, the degree of aerobic degradation after 28 days for P 1000 pellet is about 81 % for P 500 pellet is about 76 %, for P 200 is about 82 % and for P 80 is about 67 %. P 200 gives highest aerobic degradation after 28 days due to higher dissolution, degradation and low organic matter content than P 1000 and P 500.

- When considering the summation of BOD value and TCOD value after BOD as UBOD value the average degree of aerobic biodegradation for P 1000 pellet is about 83%, for P 500 is about 81% and for P 200 is about 72%. But cannot get 100% accurate results for P 1000 and P 500 due to remaining particles and fat layer after BOD test. Considering same for partial aerobic biodegradation of P 200 pellet for 14 days average degree of aerobic biodegradation for P 200 whole pellet is 66%, for P 200 grinded pellet is 40% and for P 200 autoclaved pellet is 49%.

- The average pseudo first order degradation coefficient values (k_1 value) determined using cumulative oxygen utilization figures for 28 days, for each pellet at 10 °C is as 0.0001 d^{-1} for P 1000, 0.0003 d^{-1} for P 500, 0.09 d^{-1} for P 200 and 0.08 d^{-1} for P 80. Average rate constant value for partially aerobic biodegrade pellet for 12 days at 15 °C for P 1000 pellet is 0.14 d^{-1}, for P 1000 grinded pellet 0.13 d^{-1}, for P 1000 grinded autoclaved pellet 0.15 d^{-1}. This is at 10 °C for 14 days, for P 200 pellet is 0.19 d^{-1}, for P 200 grinded pellet 0.22 d^{-1}, for P 200 grinded autoclaved pellet is 0.25 d^{-1}. k_1 value increment occurs with grinding and further increase with autoclavation suggest with increasing the temperature (summer) of water increase the rate of biodegradation due to stimulation hydrolysis process. In 12-14 days k_1 value is higher in pellets than considering 28 days complete aerobic biodegradation process due to rate of usage of organic matter higher around 12-14 days. Higher k_1 value means completion of aerobic degradation occur quickly. k_1 values suggest completion of aerobic degradation after 28 days occur as P 200> P 80> P 500> P 1000 and contribute more by P 1000 and P 500 for the negative environmental damage by releasing feed particles with more nutrients without completion of aerobic degradation.

- Average k_1 value for partially aerobic biodegrade pellet for 12 days at 15 °C for fish waste solution is 0.19 d^{-1} due to with dissolution make it easy for bacteria to biodegrade than a whole pellet. These k_1 values can be used to develop a model to determine up to which level the aerobic biodegradation occur for pellets and fish waste in marine fjord system.

- At the beginning the settling velocity of the pellets vary as P 1000>P 200>P 500 = P80 for 1m distance, velocity (ms^{-1}) gives as 0.11>0.10>0.08, 0.08. Comparatively P 1000 pellet degrade mostly while sinking. Degradation during settling, time taken for settling with relation to floating time affect for the degree of aerobic biodegradation and kinetics.

- Fish use to eat the pellets without degrade into particles. Shaking table experiment suggest lots of P 1000 and P 500 pellets remain as uneaten due to the high degradation into particles, causing negative environmental effect to enrich nutrient content in water. This can be avoided by using eatable coating layer around the particle, using attractants without using high amount of fat spray over the pellet.

- But, the pellet dissolution rate, degradation while settling the pellets, physical characteristics of each pellets as hardness affect for the degree and kinetics of aerobic degradation of each pellet in marine condition by physically degrading pellet into particles. This disaggregation provides higher surface area and accessibility for bacteria to aerobic degradation than whole pellet. But organic matter content of pellets can make a big impact for kinetics and degree of aerobic degradation than these. Even though disaggregation helps for increasing kinetics and degree of aerobic degradation in bigger pellets, due to less organic matter content and higher dissolution in P 200 pellet give higher kinetics and degree of aerobic biodegradation in fjord system.

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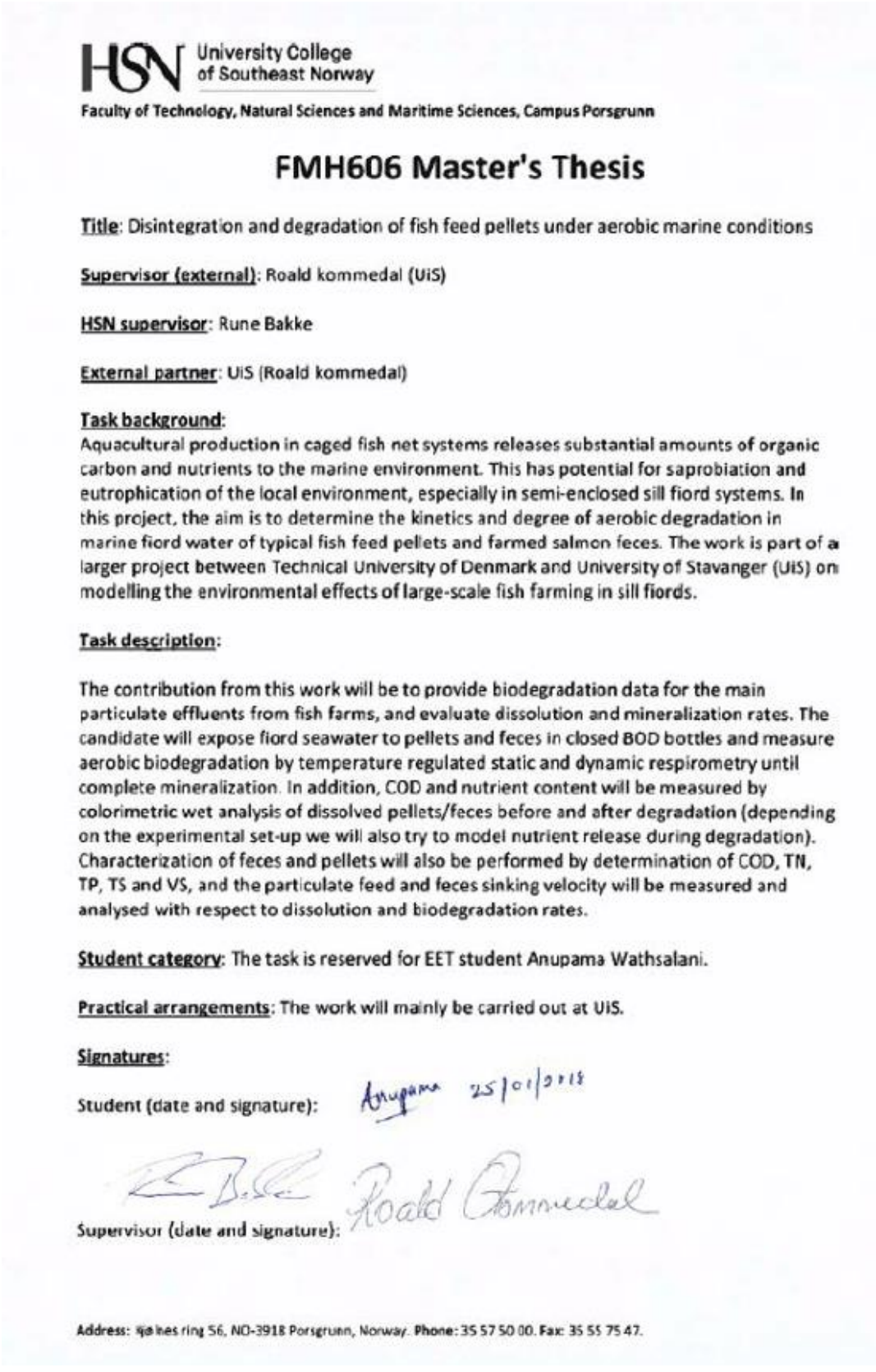
Appendices

Appendix A: Thesis summary/description page



HSN University College of Southeast Norway

Faculty of Technology, Natural Sciences and Maritime Sciences, Campus Porsgrunn

FMH606 Master's Thesis

Title: Disintegration and degradation of fish feed pellets under aerobic marine conditions

Supervisor (external): Roald kommedal (UiS)

HSN supervisor: Rune Bakke

External partner: UiS (Roald kommedal)

Task background:
Aquacultural production in caged fish net systems releases substantial amounts of organic carbon and nutrients to the marine environment. This has potential for saprobiation and eutrophication of the local environment, especially in semi-enclosed sill fiord systems. In this project, the aim is to determine the kinetics and degree of aerobic degradation in marine fiord water of typical fish feed pellets and farmed salmon feces. The work is part of a larger project between Technical University of Denmark and University of Stavanger (UiS) on modelling the environmental effects of large-scale fish farming in sill fiords.

Task description:

The contribution from this work will be to provide biodegradation data for the main particulate effluents from fish farms, and evaluate dissolution and mineralization rates. The candidate will expose fiord seawater to pellets and feces in closed BOD bottles and measure aerobic biodegradation by temperature regulated static and dynamic respirometry until complete mineralization. In addition, COD and nutrient content will be measured by colorimetric wet analysis of dissolved pellets/feces before and after degradation (depending on the experimental set-up we will also try to model nutrient release during degradation). Characterization of feces and pellets will also be performed by determination of COD, TN, TP, TS and VS, and the particulate feed and feces sinking velocity will be measured and analysed with respect to dissolution and biodegradation rates.

Student category: The task is reserved for EET student Anupama Wathsalani.

Practical arrangements: The work will mainly be carried out at UiS.

Signatures:

Student (date and signature): Anupama 25/01/2018

Supervisor (date and signature): Roald Kommedal

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Appendix B: Images related to experimental methods

Figure B-1: Fish pellets samples

Figure B-2: Electrical agitator

Figure B-3: Fish waste sample after putting into muffle furnace (VS)

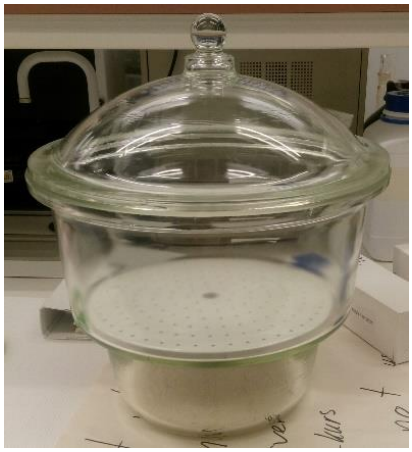


Figure B-4: Desiccator

Figure B-5: Muffle Furness

Figure B-6: COD cells

Figure B-7: Pharo 30 Spectro-quant (Darmstadt, Germany)

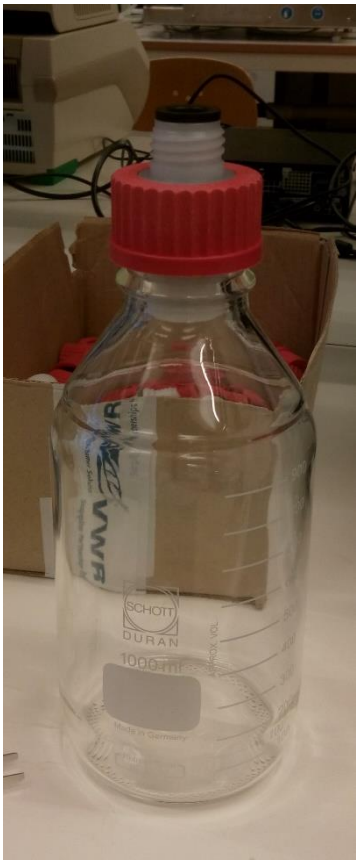


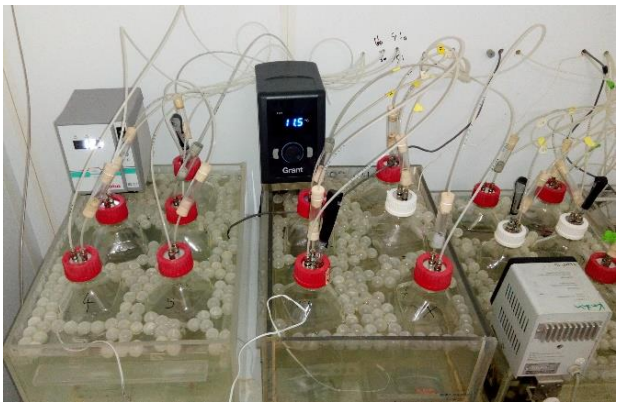
Figure B-8: NaOH granule insert part in bottle

Figure B-9: Stirring platforms inside an incubation cabinet

Figure B-10: Bottles on stirring platforms inside an incubation cabinet

Figure B-11: Measuring heads

Figure B-12: Controller



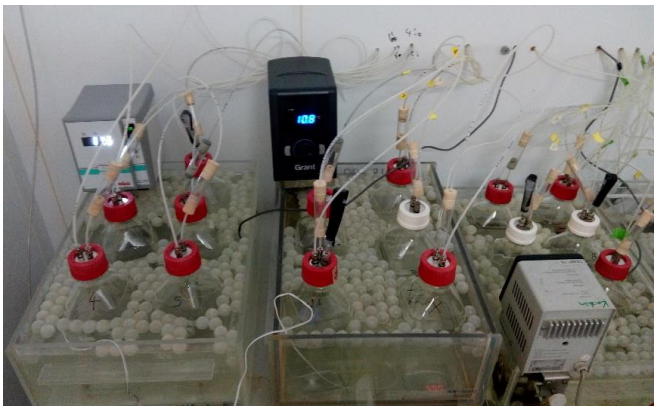
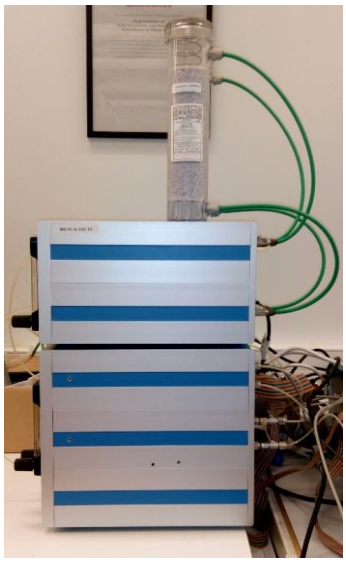


Figure B-13: Chamber arrangement in water bath



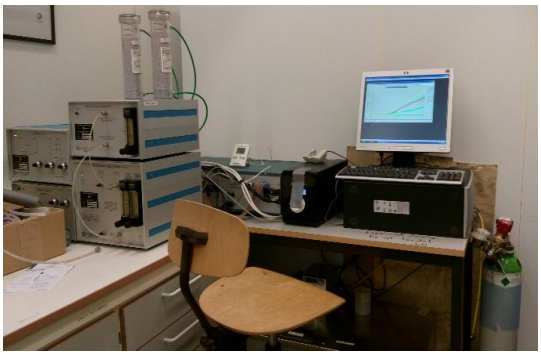


Figure B-14: Sample pump, sample dryer and data collection system for Micro-Oxymax

Figure B-15: Samples (after BOD) after putting into muffle furnace

Figure B-16: Filter papers with retain particles (after BOD) after putting into muffle furnace

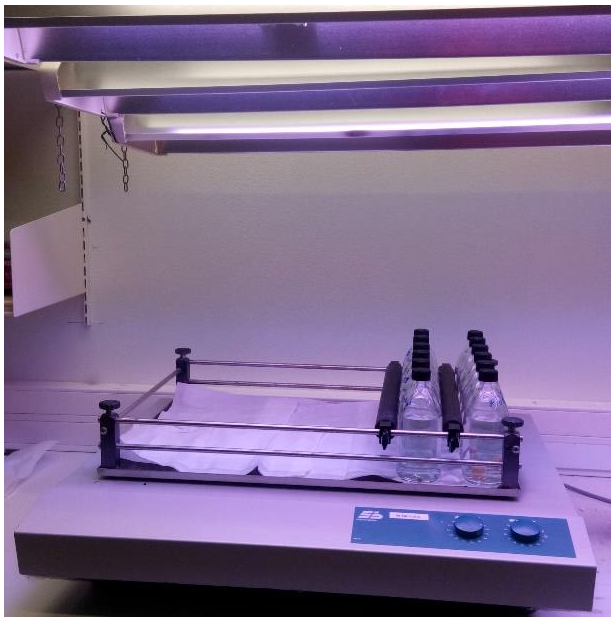


Figure B-18: Shaking table

Figure B-17: Plexiglass settling column filled with cooled (5 °C) nonfiltered sea water

Figure B-18: Autoclave machine

Appendix C: Composition of each fish pellet

Table C-1: Composition of each fish pellet

Pellet name	Protein %	Fat %
HP 1000 54 500	37.7	34.8
Ewos 500 S1 50A	38.8	31.9
Ewos 200 S1	41.9	30.7
Adapt Marine 80	44.6	28

Appendix D: Calculation of amount of fish waste in 1L of sludge solution

$$TVS = TS - TFS = 5.88 - 1.32 = 4.56\frac{g}{100g} = 45.6\ mg/g \quad \text{(C.1)}$$

$$ThOD = V_{O_2\ Fish\ feces} * \text{TVS \%} = V_{O_2\ Fish\ feces} * 0.046\ \text{g TVS/g sludge} \quad \text{(C.2)}$$

$$CTOD\ of\ for\ 1\ g\ feces\ sample = V_{O_2\ Fish\ feces} * 0.046\ \text{g TVS/g sludge} \quad \text{(C.3)}$$

$$Weight\ of\ the\ feces\ in\ the\ solution\ take\ for\ analysis = xg \quad \text{(C.4)}$$

$$COD\ for\ \ 1\ L\ solution\ with\ X\ g = 60239.43\ \text{m}gO_2 = 60.24\ gO_2 \quad \text{(C.5)}$$

$$Expected\ COD\ or\ CTOD\ for\ 1L\ solution = V_{O_2\ Fish\ feces} * 0.046\ \text{g}\frac{\text{TVS}}{\text{g}}\text{sludge} * xg \quad \text{(C.6)}$$

$$V_{O_2\ Fish\ sludge} * 0.046\ \text{g}\frac{\text{TVS}}{\text{g}}\text{sludge} * xg = 60.24\ gO_2 \quad \text{(C.7)}$$

$$V_{O_2\ Fish\ sludge} * xg = \frac{60.24}{0.046} = 1310\ gO_2 \quad \text{(C.8)}$$

According to equation (4.3), (4.8) and (4.11) assuming specific theoretical oxygen demand is same for each compound as in feed, Compound percentage taken from Table 2-2.

$$V_{O_2\ Fish\ sludge} = 2.7 * 0.11 + 1.6 * 0.26 + 1.1 * 0.36 = 1.12g\ \frac{O_2}{g\ TVS} \quad \text{(5.9)}$$

$$xg = \frac{1309.57}{1.12} = 1169\ g \quad \text{(5.10)}$$

Generally, this gives the idea of fish waste weight in 1L of solution. This explain how the initial TCOD value is high in fish waste solution with high amount of fish waste (mainly feces) in sludge sample

Appendix E: Micro-Oxymax experiment 1 related graphs

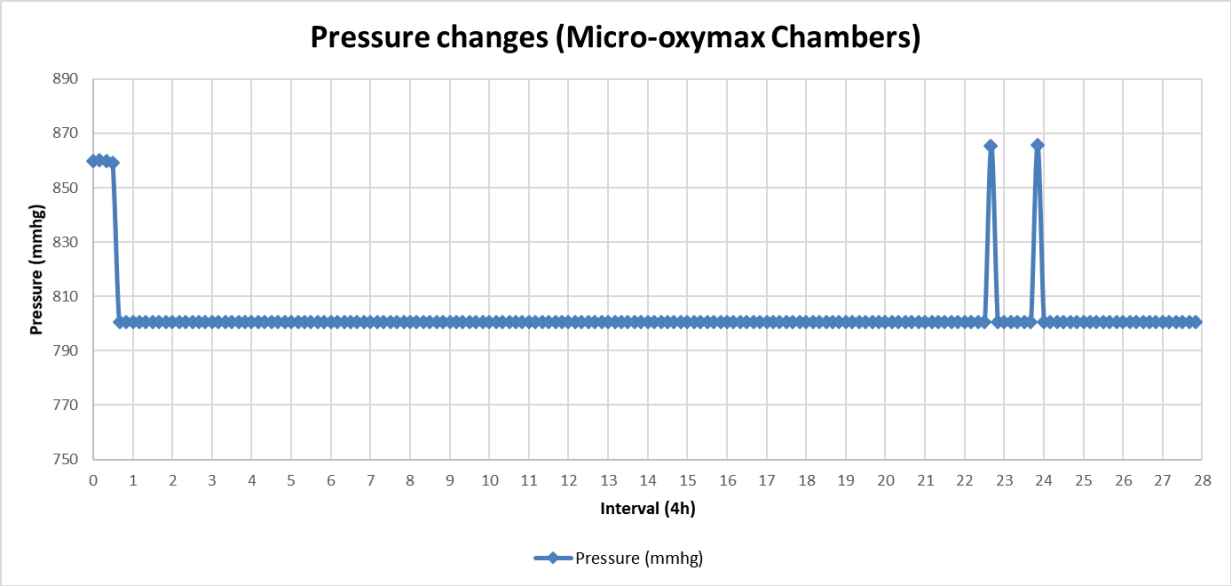


Figure E-1: Pressure changes (Micro-Oxymax Chambers)

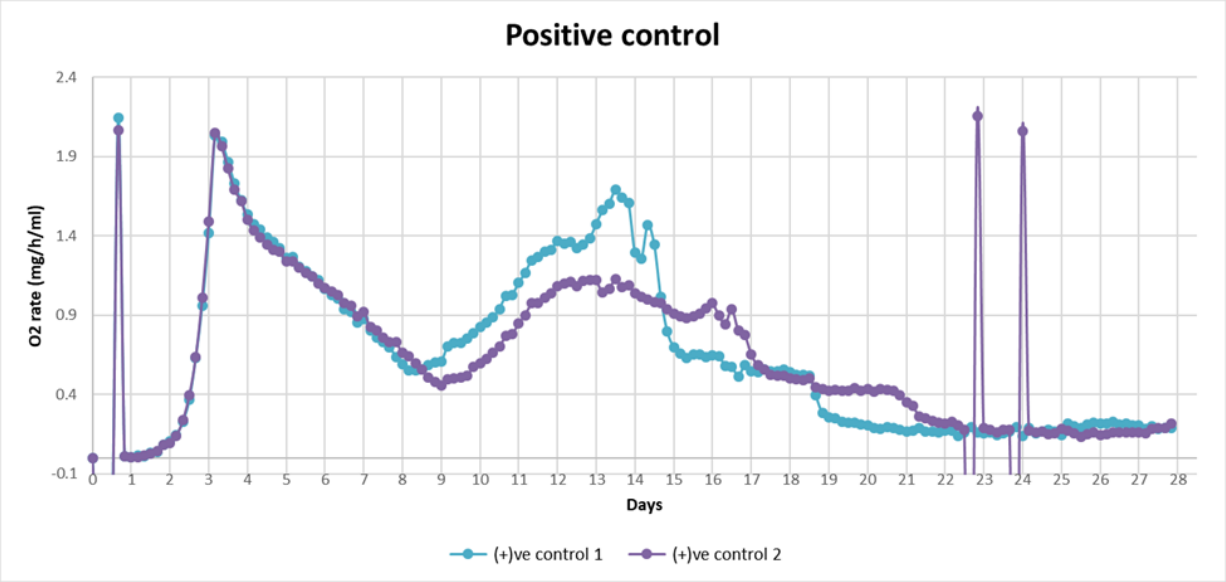


Figure E-2: O_2 utilization rate of positive controls in Micro-Oxymax test 1

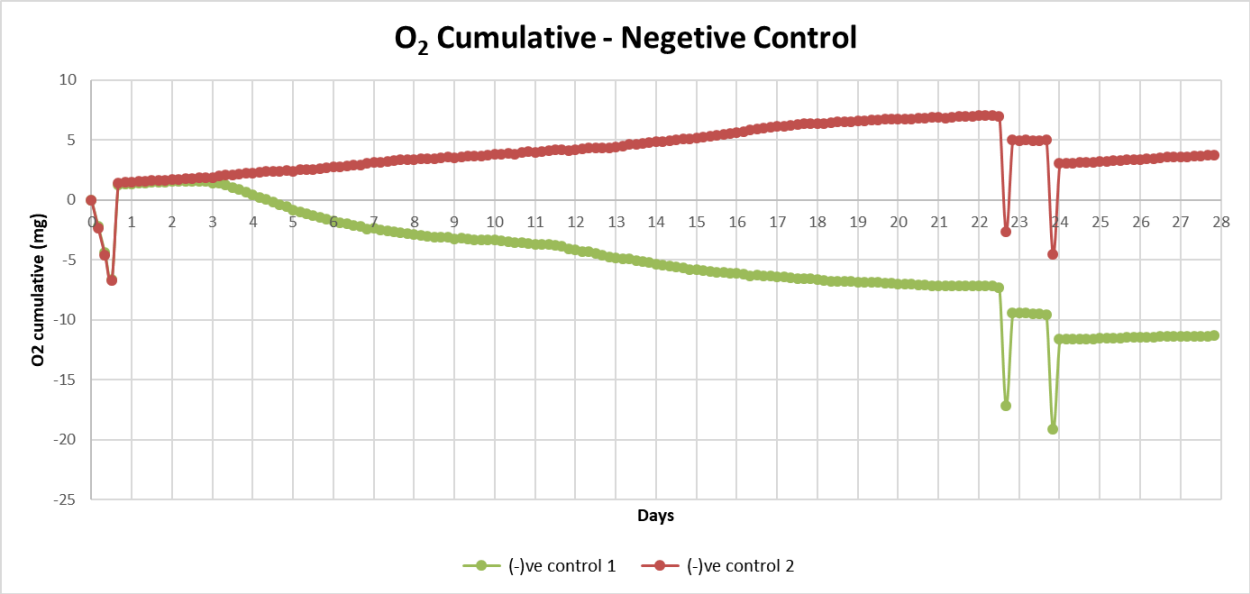


Figure E-3: Cumulative O_2 utilization of Negative controls in Micro-Oxymax test 1

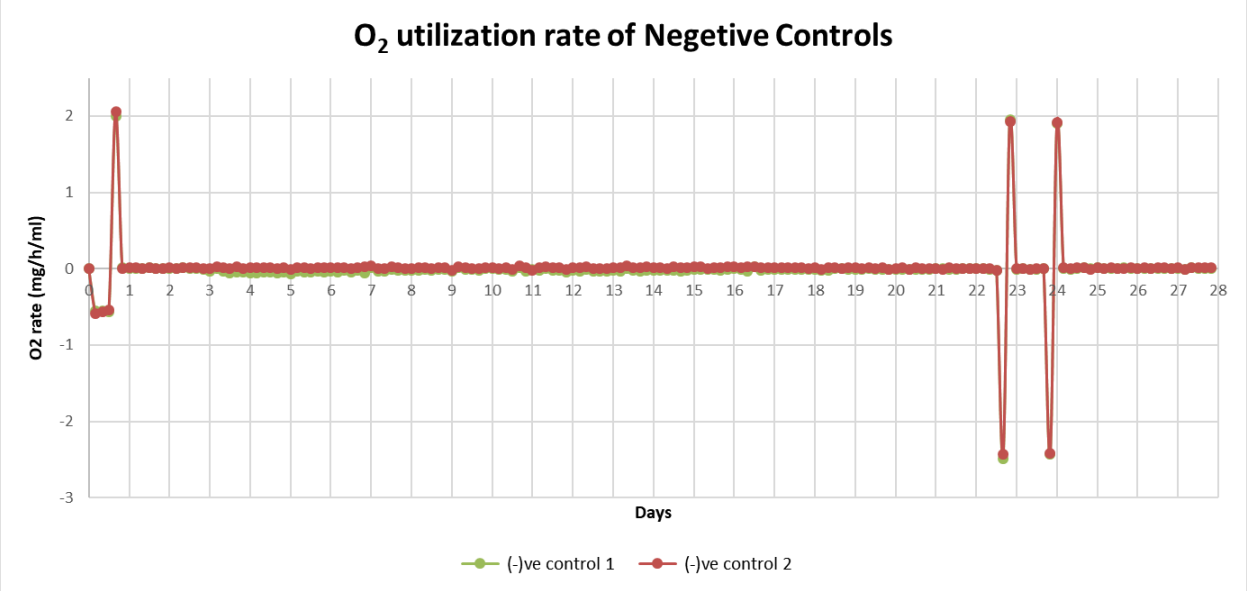


Figure E-4: O_2 utilization rate of negative controls in Micro-Oxymax test 1

Appendix F: Oxi-Top experiment 1 related graphs

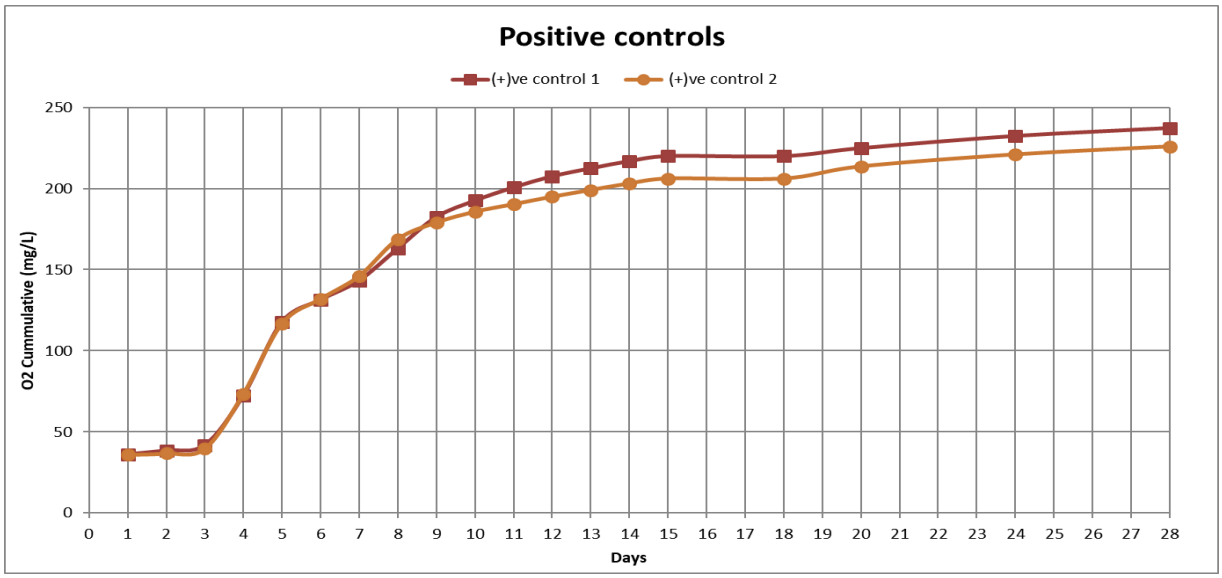


Figure F-1: Cumulative O_2 utilization of positive control in OxiTop test 1

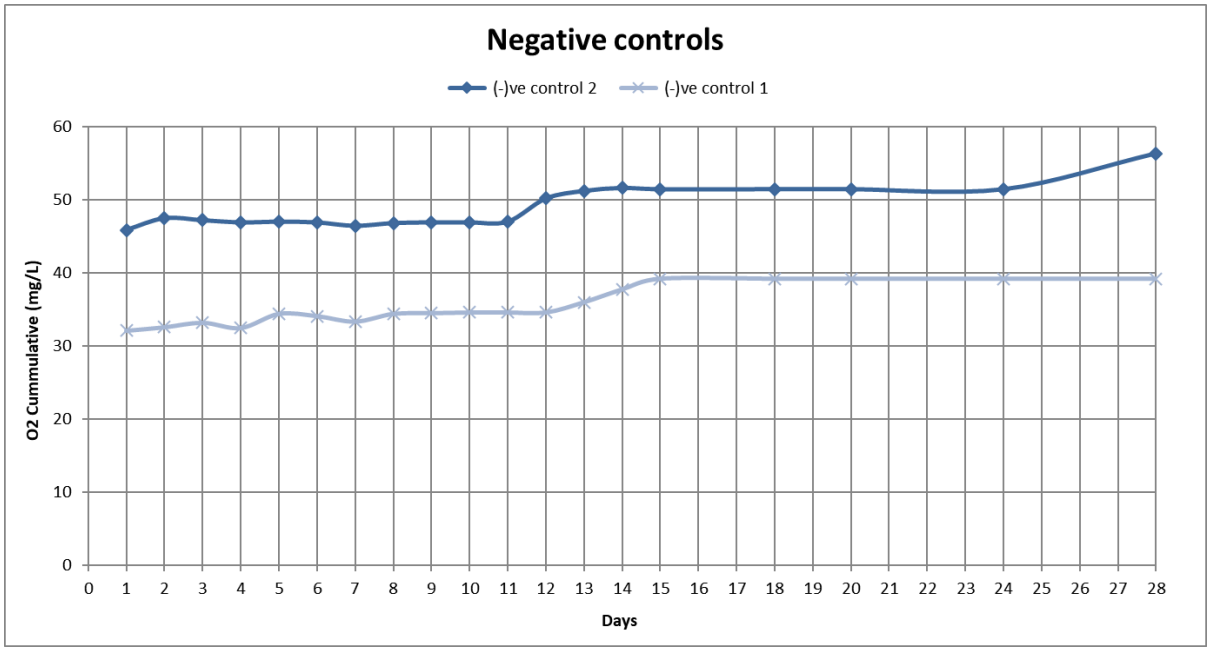


Figure F-2: Cumulative O_2 utilization of Negative control in OxiTop test 1

Appendix G: Micro-Oxymax experiment 2 related graphs

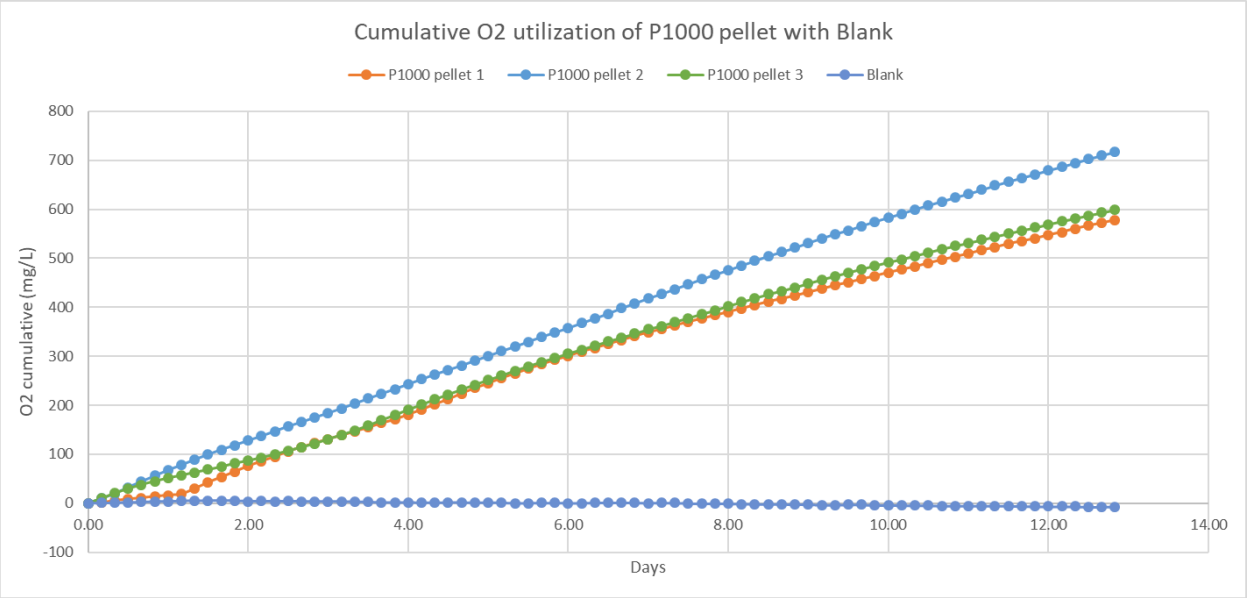


Figure G-1: Cumulative O_2 utilization of P1000 pellets in Micro-Oxymax test 2

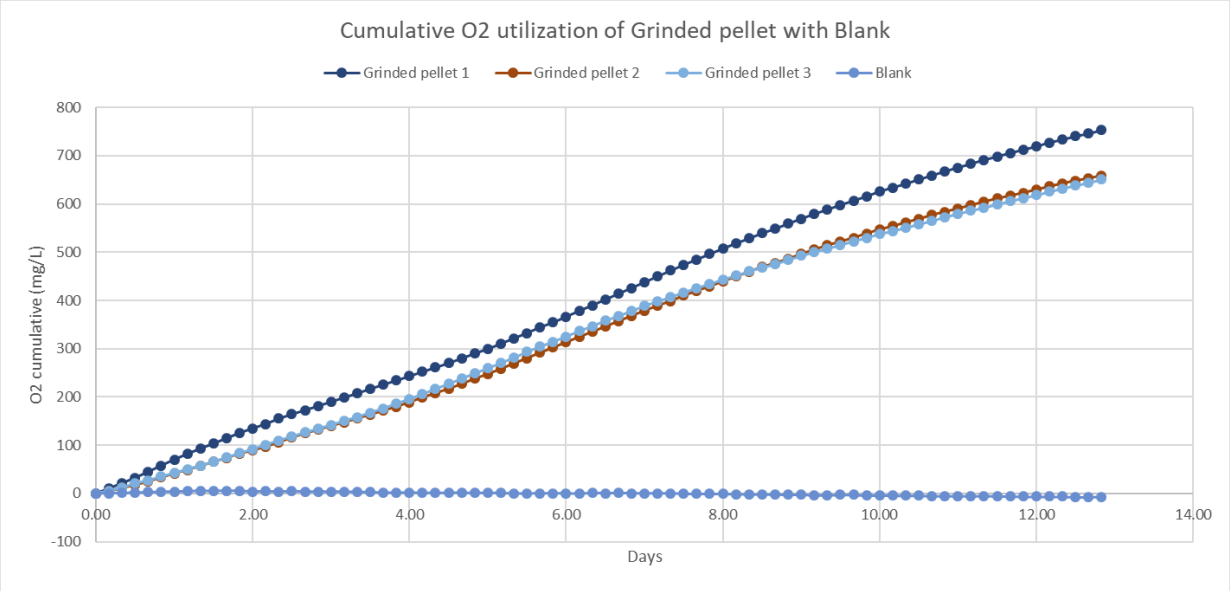


Figure G-2: Cumulative O_2 utilization of grinded pellets in Micro-Oxymax test 2

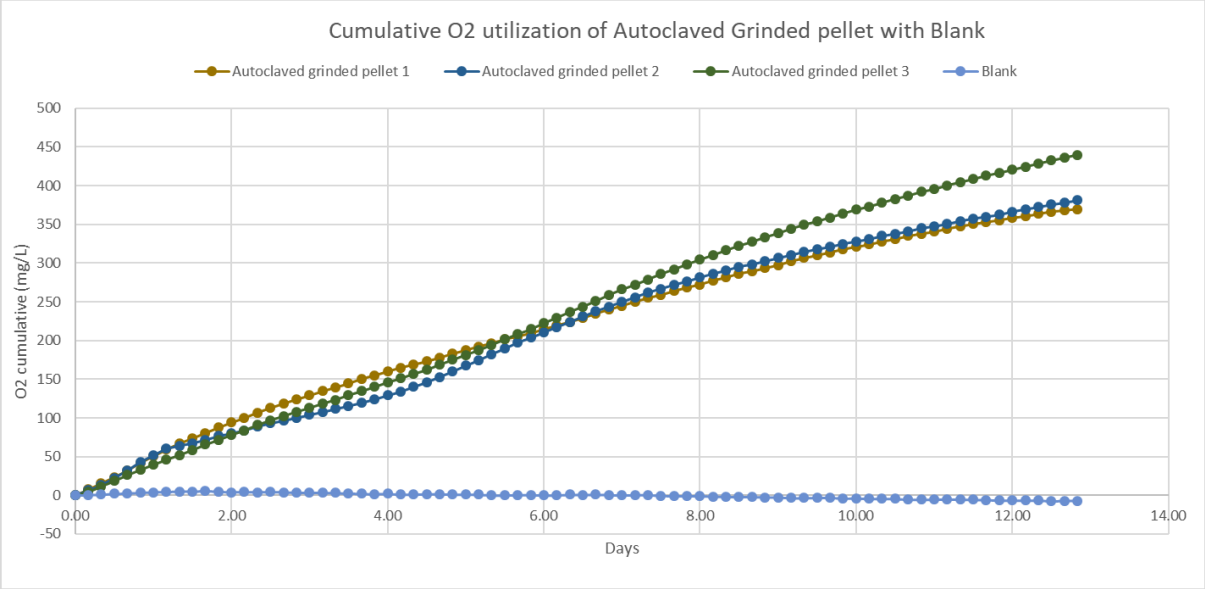


Figure G-3: Cumulative O_2 utilization of autoclaved grinded pellets in Micro-Oxymax test 2

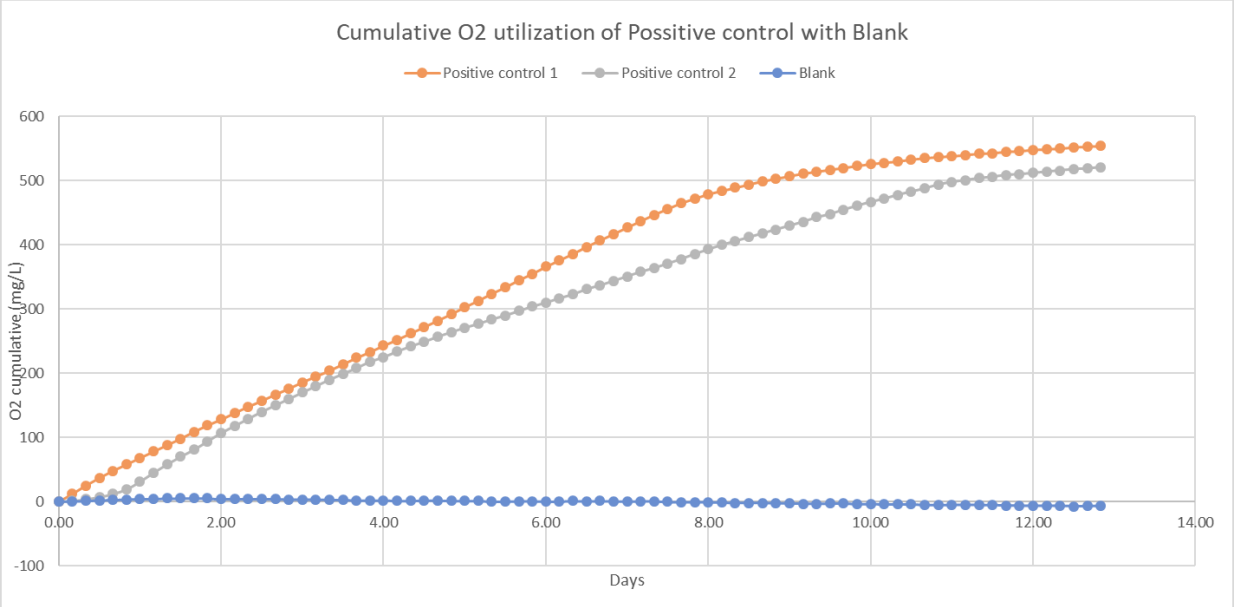


Figure G-4: Cumulative O_2 utilization of positive control in Micro-Oxymax test 2

Appendix H: Oxi-Top experiment 2 related graphs

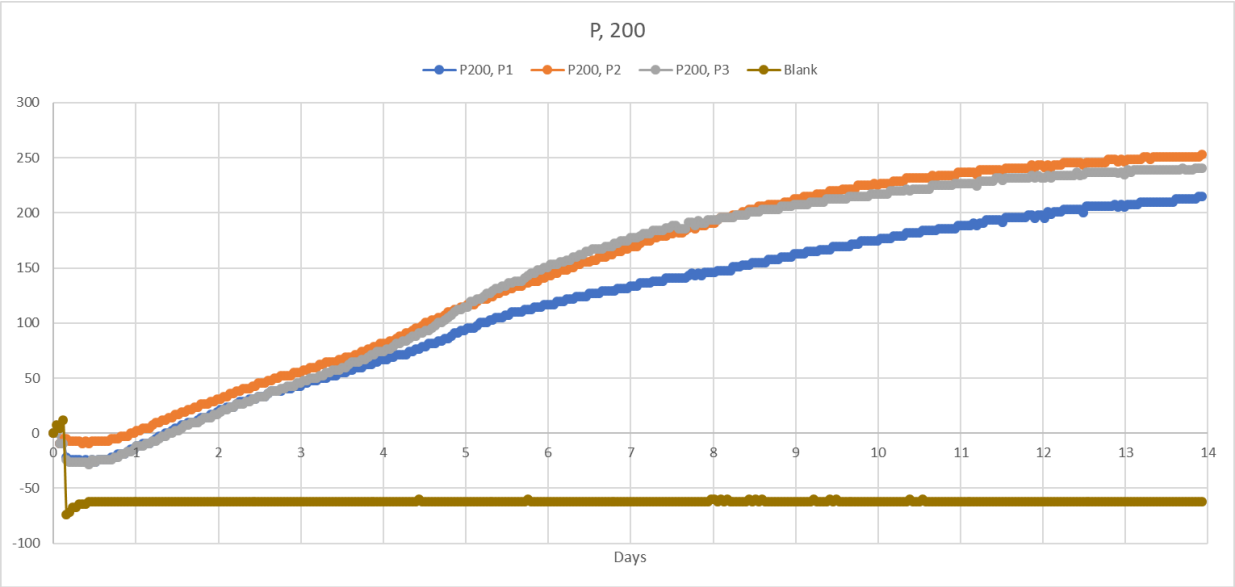


Figure H-1: Cumulative O_2 utilization of P, 200 pellets in OxiTop test 2

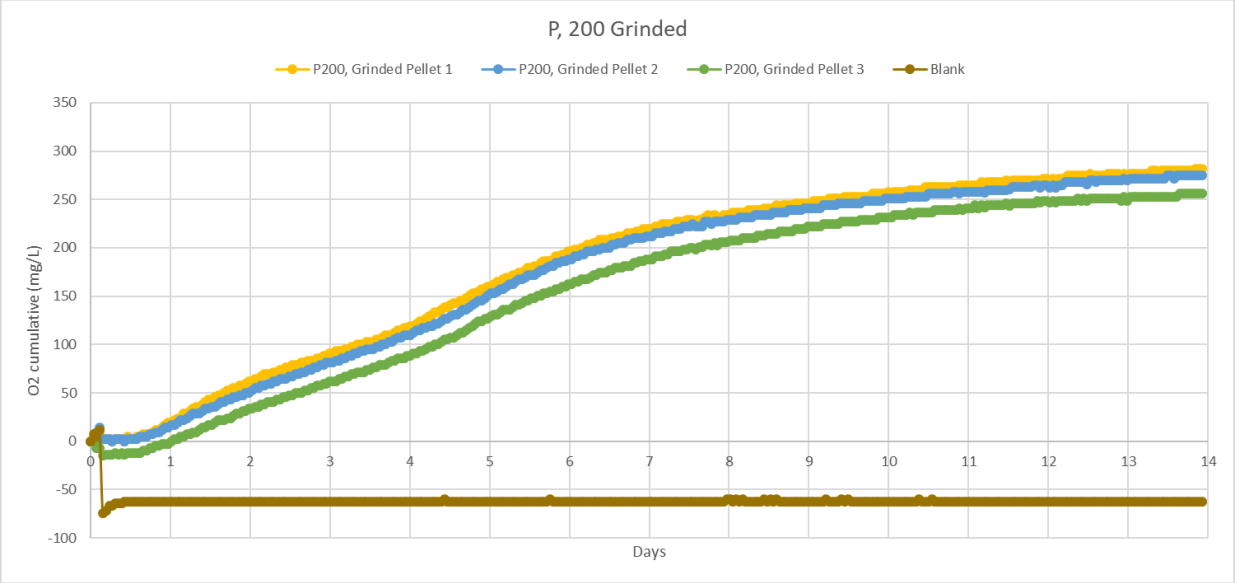


Figure H-2: Cumulative O_2 utilization of P, 200 grinded pellets in OxiTop test 2

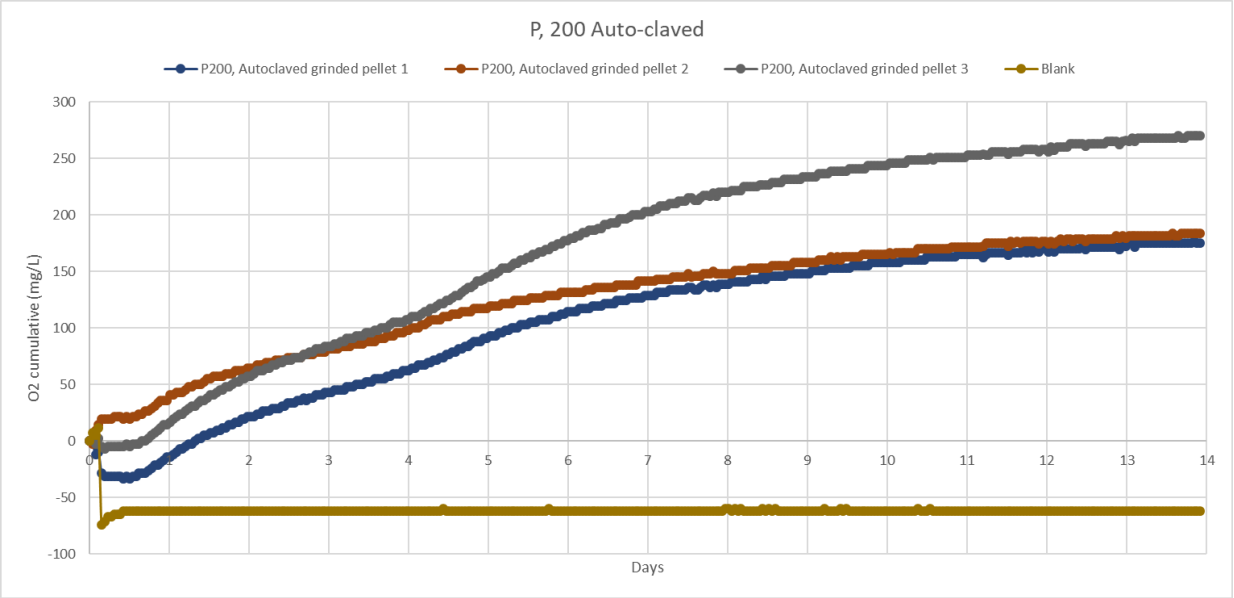


Figure H-3: Cumulative O_2 utilization of P, 200 Auto-claved pellets in OxiTop test 2

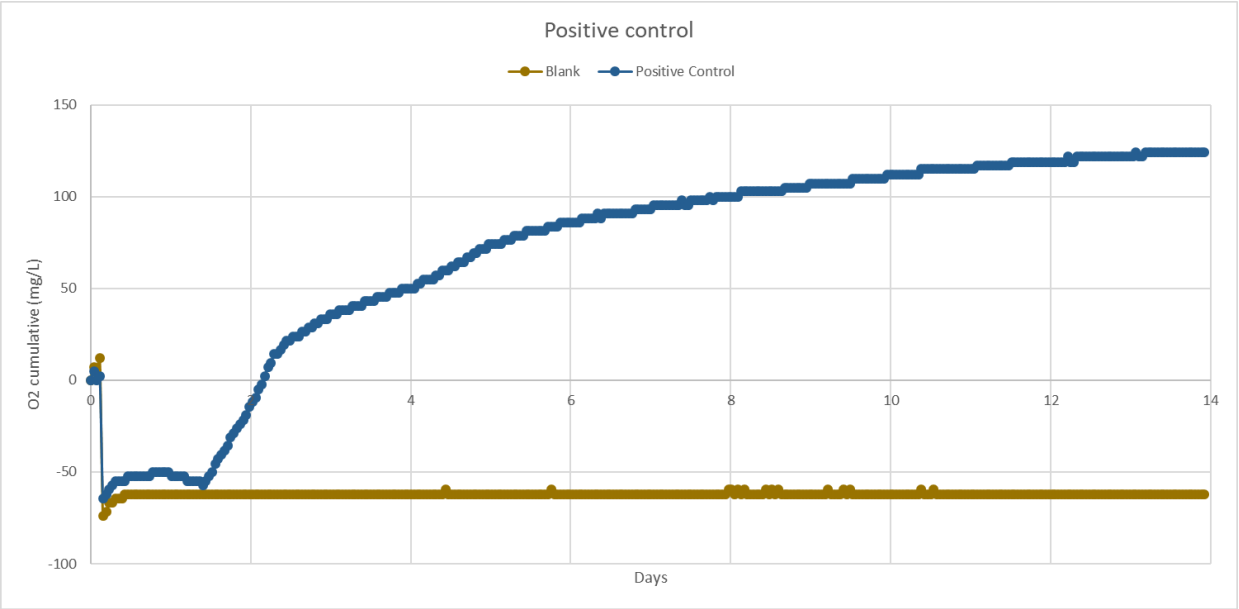


Figure H-4: Cumulative O_2 utilization of positive control in OxiTop test 2

Appendix I: Results of chemical analysis of each pellet`s solution after BOD test 1

Chemical analysis

Dates: 16,17,18 Feb 2018

Table I-1: Results of chemical Analysis of each pellet`s solution after BOD test

		TCOD					SCOD		PO4					TP					NH4					NO3		TN					Ph
Chamber number	Pellet/ Sample	COD Range (mg/L)	Value (mg/L) and dilution	Value in solution (mg/L)	Average (mg/L)	Std. Error	Value (mg/L) and dilution	Value in solution (mg/L)	PO4 Range (mg/L)	Value (mg/L) and dilution	Value in solution (mg/L)	Average (mg/L)	Std. Error	TP Range (mg/L)	Value (mg/L) and dilution	Value in solution (mg/L)	Average (mg/L)	Std. Error	NH4 Range (mg/L)	Value (mg/L) and dilution	Value in solution (mg/L)	Average (mg/L)	Std. Error	NO3 Range (mg/L)	Value in original solution (mg/L)	TN Range (mg/L)	Value (mg/L) and dilution	Value in solution (mg/L)	Average (mg/L)	Std. Error	
Microoxy max																															
1	P 1000,1	4-40	19.9 (1:10)	199	197	2	12.3 (1:10)	123	0.05 - 5	0.83 (1:10)	8.3	8.45	0.15	0.05 - 5	0.94 (1:10)	9.4	9.35	0.05	0.5-16	2.7 (1:10)	27	27.5	0.5	0.5-18	<0.5	0.5-15	3.3 (1:10)	33	32	1	
			19.5 (1:10)	195						0.86 (1:10)	8.6				0.93 (1:10)	9.3				2.8 (1:10)	28						3.1 (1:10)	31			
2	P 1000,2	4-40	25.5 (1:10)	255	245.5	9.5	12.9 (1:10)	129	0.05 - 5	0.74 (1:10)	7.4	7.5	0.1	0.05 - 5	0.89 (1:10)	8.9	8.95	0.05	0.5-16	2.2 (1:10)	22	22.5	0.5	0.5-18	<0.5	0.5-15	2.6 (1:10)	26	26	0	
			23.6 (1:10)	236						0.76 (1:10)	7.6				0.9 (1:10)	9				2.3 (1:10)	23						2.6 (1:10)	26			
3	P 1000,3	4-40	23 (1:10)	230	252	22	12.5 (1:10)	125	0.05 - 5	0.83 (1:10)	8.3	8.3	0	0.05 - 5	1.08 (1:10)	10.8	10.65	0.15	0.5-16	2.6 (1:10)	26	25.5	0.5	0.5-18	<0.5	0.5-15	3.5 (1:10)	35	35.5	0.5	7.048
			27.4 (1:10)	274						0.83 (1:10)	8.3				1.05 (1:10)	10.5				2.5 (1:10)	25						3.6 (1:10)	36			
4	P 1000,4	4-40	17.4 (1:10)	174	174		12.6 (1:10)	126	0.05 - 5	0.89 (1:10)	8.9	8.9	0	0.05 - 5	0.97 (1:10)	9.7	9.7	0	0.5-16	3.2 (1:10)	32	31	1	0.5-18	<0.5	0.5-15	3.7 (1:10)	37	36.5	0.5	
										0.89 (1:10)	8.9				0.97 (1:10)	9.7				3 (1:10)	30						3.6 (1:10)	36			
5	P 500, 1	4-40	11.7 (1:10)	117	117		6.6 (1:10)	66	0.05 - 5	0.69 (1:10)	6.9	6.85	0.05	0.05 - 5	0.79 (1:10)	7.9	7.8	0.1	0.5-16	1.3 (1:10)	13	13	0	0.5-18	<0.5	0.5-15	1.8 (1:10)	18	17.5	0.5	
										0.68 (1:10)	6.8				0.77 (1:10)	7.7				1.3 (1:10)	13						1.7 (1:10)	17			
6	P 500, 2	4-40	14.3 (1:10)	143	143		7.3 (1:10)	73	0.05 - 5	0.66 (1:10)	6.6	6.6	0	0.05 - 5	0.74 (1:10)	7.4	7.35	0.05	0.5-16	1.8 (1:10)	18	18	0	0.5-18	<0.5	0.5-15	2.3 (1:10)	23	22	1	
										0.66 (1:10)	6.6				0.73 (1:10)	7.3				1.8 (1:10)	18						2.1 (1:10)	21			
7	P 500, 3	4-40	14.7 (1:10)	147	147		9 (1:10)	90	0.05 - 5	0.81 (1:10)	8.1	8.15	0.05	0.05 - 5	0.9 (1:10)	9	8.95	0.05	0.5-16	1.8 (1:10)	18	18.5	0.5	0.5-18	<0.5	0.5-15	2.4 (1:10)	24	23.5	0.5	7.084
										0.82 (1:10)	8.2				0.89 (1:10)	8.9				1.9 (1:10)	19						2.3 (1:10)	23			
8	P 500, 4	4-40	10.4 (1:10)	104	104		6.5 (1:10)	65	0.05 - 5	0.57 (1:10)	5.7	5.7	0	0.05 - 5	0.61 (1:10)	6.1	6.1	0	0.5-16	1.4 (1:10)	14	14	0	0.5-18	<0.5	0.5-15	1.7 (1:10)	17	17	0	
										0.57 (1:10)	5.7				0.61 (1:10)	6.1				1.4 (1:10)	14						1.7 (1:10)	17			
Oxitop																															
1	P 200, 1	4-40	9.7 (1:10)	97	97		6.4 (1:10)	64	0.05 - 5	0.43 (1:10)	4.3	4.35	0.05	0.05 - 5	0.53 (1:10)	5.3	5.25	0.05	0.5-16	1.4 (1:10)	14	14	0	0.5-18	<0.5	0.5-15	1.6 (1:10)	16	16	0	
										0.44 (1:10)	4.4				0.52 (1:10)	5.2				1.4 (1:10)	14						1.6 (1:10)	16			
2	P 200, 2	4-40	14.9 (1:10)	149	149		5.5 (1:10)	55	0.05 - 5	0.44 (1:10)	4.4	4.45	0.05	0.05 - 5	0.57 (1:10)	5.7	5.9	0.2	0.5-16	1.3 (1:10)	13	13.5	0.5	0.5-18	<0.5	0.5-15	1.6 (1:10)	16	15	1	
										0.45 (1:10)	4.5				0.61 (1:10)	6.1				1.4 (1:10)	14						1.4 (1:10)	14			
3	P 200, 3	4-40	6.9 (1:10)	69	69		5.7 (1:10)	57	0.05 - 5	0.43(1:10)	4.3	4.30	0	0.05 - 5	0.5 (1:10)	5	5.95	0.95	0.5-16	1.5 (1:10)	15	15	0	0.5-18	<0.5	0.5-15	1.6 (1:10)	16	17.5	1.5	7.883
										0.43 (1:10)	4.3				0.69 (1:10)	6.9				1.5 (1:10)	15						1.9 (1:10)	19			
4	P 200, 4	4-40	8.1 (1:10)	81	81		6.4 (1:10)	64	0.05 - 5	0.47 (1:10)	4.7	4.6	0.1	0.05 - 5	0.52 (1:10)	5.2	5.85	0.65	0.5-16	1.4 (1:10)	14	14	0	0.5-18	<0.5	0.5-15	1.4 (1:10)	14	15.5	1.5	
										0.45 (1:10)	4.5				0.65 (1:10)	6.5				1.4 (1:10)	14						1.7 (1:10)	17			
5	P 80, 1	4-40							0.05 - 5	0.36 (1:10)	3.6	3.65	0.05	0.05 - 5	0.51	5.1	5.05	0.05	0.5-16	0.9 (1:10)	9	9	0	0.5-18	<0.5	0.5-15	1.3 (1:10)	13	12.5	0.5	
										0.37 (1:10)	3.7				0.5	5				0.9 (1:10)	9						1.2 (1:10)	12			
6	P 80, 2	4-40							0.05 - 5	0.38 (1:10)	3.8	3.8	0	0.05 - 5	0.52	5.2	4.9	0.3	0.5-16	1 (1:10)	10	10	0	0.5-18	<0.5	0.5-15	1.4 (1:10)	14	13.5	0.5	
										0.38 (1:10)	3.8				0.46	4.6				1 (1:10)	10						1.3 (1:10)	13			
7	P 80, 3	4-40							0.05 - 5	0.36 (1:10)	3.6	3.6	0	0.05 - 5	0.51	5.1	4.9	0.2	0.5-16	1 (1:10)	10	10	0	0.5-18	<0.5	0.5-15	1.3 (1:10)	13	13	0	8.128
										0.36 (1:10)	3.6				0.47	4.7				1 (1:10)	10						1.3 (1:10)	13			
8	P 80, 4	4-40							0.05 - 5	0.38 (1:10)	3.8	3.75	0.05	0.05 - 5	0.42	4.2	4.2	0	0.5-16	0.9 (1:10)	9	9	0	0.5-18	<0.5	0.5-15	1.2 (1:10)	12	12	0	
										0.37 (1:10)	3.7				0.42	4.2				0.9 (1:10)	9						1.2 (1:10)	12			

n

TS/TFS/VS

Dates: 16,17,18 Feb 2018

Table I-2: Results of TS/TFS/VS analysis of each pellet`s solution after BOD test

Chamber number	Pellet/ Sample	Initial wgt of crusible	Sample Volume (mL)	Crusible+ sample (24h Oven) (g)	Crusible+Sample (Muffle furnes) g	Sample weight after oven dry (g)	Sample weight after Muffel furnes (g)	TS (g/L)	Average TS (g/L)	Std. Error TS	TFS (g/L)	Average TFS (g/L)	Std. Error TFS	VS (g/L)	Average VS (g/L)	Std. Error VS
Microoxy max																
1	P 1000,1	47.5582	15	48.1224	48.0123	0.5642	0.4541	37.6133	37.1217	0.4917	30.2733	29.8717	0.4017	7.3400	7.2500	0.0900
		22.2662	10	22.6325	22.5609	0.3663	0.2947	36.6300			29.4700			7.1600		
2	P 1000,2	17.6132	10	17.9924	17.9205	0.3792	0.3073	37.9200	37.6200	0.3000	30.7300	30.5450	0.1850	7.1900	7.0750	0.1150
		48.3477	15	48.9075	48.8031	0.5598	0.4554	37.3200			30.3600			6.9600		
3	P 1000,3	17.2192	10	17.7562	17.5236	0.5370	0.3044	53.7000	46.8900	6.8100	30.4400	30.3450	0.0950	23.2600	16.5450	6.7150
		15.3015	10	15.7023	15.6040	0.4008	0.3025	40.0800			30.2500			9.8300		
4	P 1000,4	17.013	10	17.4155	17.3164	0.4025	0.3034	40.2500	38.3750	1.8750	30.3400	30.3467	0.0067	9.9100	8.0283	1.8817
		102.8012	15	103.3487	103.2565	0.5475	0.4553	36.5000			30.3533			6.1467		
5	P 500, 1	32.1369	10	32.5121	32.4429	0.3752	0.3060	37.5200	37.3100	0.2100	30.6000	30.6000	0.0000	6.9200	6.7100	0.2100
		90.0786	15	90.6351	90.5376	0.5565	0.4590	37.1000			30.6000			6.5000		
6	P 500, 2	30.9538	10	31.3295	31.2559	0.3757	0.3021	37.5700	37.7317	0.1617	30.2100	30.4550	0.2450	7.3600	7.2767	0.0833
		86.6077	15	87.1761	87.0682	0.5684	0.4605	37.8933			30.7000			7.1933		
7	P 500, 3	16.8375	10	17.3305	17.1454	0.4930	0.3079	49.3000	45.0150	4.2850	30.7900	30.4750	0.3150	18.5100	14.5400	3.9700
		16.7535	10	17.1608	17.0551	0.4073	0.3016	40.7300			30.1600			10.5700		
8	P 500, 4	17.1664	10	17.5997	17.4690	0.4333	0.3026	43.3300	42.9850	0.3450	30.2600	30.0500	0.2100	13.0700	12.9350	0.1350
		17.2499	10	17.6763	17.5483	0.4264	0.2984	42.6400			29.8400			12.8000		
Oxitop																
3	P 200, 1	31.3084	10.0000	31.6838	31.6093	0.3754	0.3009	37.5400	37.4400	0.1000	30.0900	30.2200	0.1300	7.4500	7.2200	0.2300
		31.8527	10	32.2261	32.1562	0.3734	0.3035	37.3400			30.3500			6.9900		
4	P 200, 2	16.9484	10	17.3986	17.2501	0.4502	0.3017	45.0200	42.3800	2.6400	30.1700	29.9300	0.2400	14.8500	12.4500	2.4000
		16.7529	10	17.1503	17.0498	0.3974	0.2969	39.7400			29.6900			10.0500		
7	P 200, 3	17.1759	10	17.6171	17.4789	0.4412	0.3030	44.1200	41.1500	2.9700	30.3000	30.3100	0.0100	13.8200	10.8400	2.9800
		17.3135	10	17.6953	17.6167	0.3818	0.3032	38.1800			30.3200			7.8600		
8	P 200, 4	34.3766	10	34.7500	34.6756	0.3734	0.2990	37.3400	36.6550	0.6850	29.9000	29.9250	0.0250	7.4400	6.7300	0.7100
		20.2415	10	20.6012	20.5410	0.3597	0.2995	35.9700			29.9500			6.0200		
9	P 80, 1	17.2493	10	17.7056	17.5525	0.4563	0.3032	45.6300	42.4350	3.1950	30.3200	30.2500	0.0700	15.3100	12.1850	3.1250
		17.1661	10	17.5585	17.4679	0.3924	0.3018	39.2400			30.1800			9.0600		
10	P 80, 2	17.4533	10	17.8727	17.7565	0.4194	0.3032	41.9400	42.0650	0.1250	30.3200	30.2650	0.0550	11.6200	11.8000	0.1800
		17.4806	10	17.9025	17.7827	0.4219	0.3021	42.1900			30.2100			11.9800		
11	P 80, 3	16.8376	10	17.2734	17.1389	0.4358	0.3013	43.5800	55.7833	12.2033	30.1300	30.2883	0.1583	13.4500	25.4950	12.0450
		31.7833	15	32.8031	32.2400	1.0198	0.4567	67.9867			30.4467			37.5400		
12	P 80, 4	16.6154	10	17.0621	16.9164	0.4467	0.3010	44.6700	43.4300	1.2400	30.1000	30.1300	0.0300	14.5700	13.3000	1.2700
		16.9437	10	17.3656	17.2453	0.4219	0.3016	42.1900			30.1600			12.0300		

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Table I-3: Results of TSS/FSS/VSS analysis of each pellet`s solution after BOD test

TSS/FFS/VSS

Dates: 16,17,18 Feb 2018

TS	Pellet/ Sample	Initial wgt of FP (g)	Sample Volume (mL)	Crusible weight (g)	FP+ sample (24h Oven) (g)	FP +Sample (Muffle furnes) g	Weight of FP after oven dry (g)	Weight of FP after Muffel furnes (g)	Wt. of FP after Muffel furnes with weight loss correction (g)	TSS (g/L)	Average TSS (g/L)	Std. Error TSS	FSS (g/L)	Average FSS (g/L)	Std. Error FSS	VSS (g/L)	Average VSS (g/L)	Std. Error VSS
Microoxy max																		
1	P 1000,1	0.0858	20	86.6106	86.7096	86.7022	0.0990	0.0916	0.0893	0.6600	0.6950	0.0350	0.1750	0.2375	0.0625	0.4850	0.4575	0.0275
		0.0859	20	30.9563	31.0568	31.0505	0.1005	0.0942	0.0919	0.7300			0.3000			0.4300		
2	P 1000,2	0.0860	20	34.3798	34.4887	34.4805	0.1089	0.1007	0.0984	1.1450	1.1875	0.0425	0.6200	0.6625	0.0425	0.5250	0.5250	0.0000
		0.0883	20	31.8542	31.9671	31.9589	0.1129	0.1047	0.1024	1.2300			0.7050			0.5250		
3	P 1000,3	0.0876	20	22.2677	22.3678	22.3616	0.1001	0.0939	0.0916	0.6250	0.5975	0.0275	0.2000	0.1825	0.0175	0.4250	0.4150	0.0100
		0.0874	20	31.3095	31.4083	31.4025	0.0988	0.0930	0.0907	0.5700			0.1650			0.4050		
4	P 1000,4	0.0874	20	20.2427	20.3421	20.3379	0.0994	0.0952	0.0929	0.6000	0.6650	0.0650	0.2750	0.2975	0.0225	0.3250	0.3675	0.0425
		0.0886	20	47.5598	47.6630	47.6571	0.1032	0.0973	0.0950	0.7300			0.3200			0.4100		
5	P 500, 1	0.0873	20	48.3491	48.4462	48.4416	0.0971	0.0925	0.0902	0.4900	0.4775	0.0125	0.1450	0.1600	0.0150	0.3450	0.3175	0.0275
		0.0874	20	32.1374	32.2341	32.2306	0.0967	0.0932	0.0909	0.4650			0.1750			0.2900		
6	P 500, 2	0.0881	20	37.4324	37.5411	37.5365	0.1087	0.1041	0.1018	1.0300	1.0075	0.0225	0.6850	0.5700	0.1150	0.3450	0.4375	0.0925
		0.0861	20	90.0815	90.1873	90.1790	0.1058	0.0975	0.0952	0.9850			0.4550			0.5300		
7	P 500, 3	0.0853	20	102.8023	102.8979	102.8901	0.0956	0.0878	0.0855	0.5150	0.5150	0.0000	0.0100	0.1150	0.1050	0.5050	0.4000	0.1050
		0.0845	20	37.4305	37.5253	37.5217	0.0948	0.0912	0.0889	0.5150			0.2200			0.2950		
8	P 500, 4	0.0881	20	86.6064	86.7064	86.7031	0.1000	0.0967	0.0944	0.5950	0.5600	0.0350	0.3150	0.3025	0.0125	0.2800	0.2575	0.0225
		0.0884	20	31.3081	31.4070	31.4046	0.0989	0.0965	0.0942	0.5250			0.2900			0.2350		
Oxitop																		
3	P 200, 1	0.0869	20	22.2675	22.3730	22.3693	0.1055	0.1018	0.0995	0.9300	0.9200	0.0100	0.6300	0.6100	0.0200	0.3000	0.3100	0.0100
		0.0846	20	47.5590	47.6618	47.6577	0.1028	0.0987	0.0964	0.9100			0.5900			0.3200		
4	P 200, 2	0.0868	20	31.8543	31.9612	31.9573	0.1069	0.1030	0.1007	1.0050	1.0400	0.0350	0.6950	0.6675	0.0275	0.3100	0.3725	0.0625
		0.0866	20	102.8011	102.9092	102.9028	0.1081	0.1017	0.0994	1.0750			0.6400			0.4350		
7	P 200, 3	0.0866	20	90.0814	90.1765	90.1722	0.0951	0.0908	0.0885	0.4250	0.4300	0.0050	0.0950	0.1350	0.0400	0.3300	0.2950	0.0350
		0.0868	20	32.1380	32.2335	32.2306	0.0955	0.0926	0.0903	0.4350			0.1750			0.2600		
8	P 200, 4	0.0851	20	20.2426	20.3399	20.3367	0.0973	0.0941	0.0918	0.6100	0.5950	0.0150	0.3350	0.3200	0.0150	0.2750	0.2750	0.0000
		0.0860	20	31.3090	31.4066	31.4034	0.0976	0.0944	0.0921	0.5800			0.3050			0.2750		
9	P 80, 1	0.0852	20	37.4321	37.5369	37.5325	0.1048	0.1004	0.0981	0.9800	1.0025	0.0225	0.6450	0.6875	0.0425	0.3350	0.3150	0.0200
		0.0875	20	31.8537	31.9617	31.9581	0.1080	0.1044	0.1021	1.0250			0.7300			0.2950		
10	P 80, 2	0.0861	20	47.5590	47.6562	47.6514	0.0972	0.0924	0.0901	0.5550	0.5550	0.0000	0.2000	0.2450	0.0450	0.3550	0.3100	0.0450
		0.0873	20	32.1376	32.2360	32.2330	0.0984	0.0954	0.0931	0.5550			0.2900			0.2650		
11	P 80, 3	0.0870	20	20.2426	20.3398	20.3364	0.0972	0.0938	0.0915	0.5100	0.5225	0.0125	0.2250	0.2500	0.0250	0.2850	0.2725	0.0125
		0.0880	20	22.2675	22.3662	22.3633	0.0987	0.0958	0.0935	0.5350			0.2750			0.2600		
12	P 80, 4	0.0876	20	30.9554	31.0559	31.0521	0.1005	0.0967	0.0944	0.6450	0.6050	0.0400	0.3400	0.3000	0.0400	0.3050	0.3050	0.0000
		0.0850	20	48.3487	48.4450	48.4412	0.0963	0.0925	0.0902	0.5650			0.2600			0.3050		

		Initial wgt of FP (g)	Crusible weight (g)	FP+ sample (24h Oven) (g)	FP +Sample (Muffle furnes) g	Weight of FP after oven dry (g)	Weight of FP after Muffel furnes(g)	Difference after Oven (g)	Difference after Oven (g)	Average after MF
13	Blank 1	0.0869	34.3789	34.4658	34.4633	0.0869	0.0844	0	0.0025	0.0023
	Blank 2	0.0893	30.9553	31.0446	31.0425	0.0893	0.0872	0	0.0021	

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Table I-4: Relationship between TCOD with VS, SCOD with (VS-VSS) and amount of oxygen required to degradation of one gram of total, soluble and insoluble organic matter

Pellet/ Sample	TCOD in chamber (mg O_2)	SCOD in chamber (mg/L)	TS in chamber (mg O_2)	VS in chamber (g)	VSS (g)	TSS (g)	(VSS/VS) * 100	VS-VSS (g)	g O_2 for 1g soluble organic matter	g O_2 for 1g total organic matter	g $O_2$for 1g insoluble organic matter
P 1000,1	139	87	26	5	0.3	0.5	6.3	4.8	18	27	9
P 1000,2	173	91	26	5	0.4	0.8	7.4	4.6	20	35	15
P 1000,3	177	88	33	12	0.3	0.4	2.5	11.4	8	15	7
P 1000,4	122	89	27	6	0.3	0.5	4.6	5.4	16	22	5
P 500, 1	82	46	26	5	0.2	0.3	4.7	4.5	10	17	7
P 500, 2	101	51	27	5	0.3	0.7	6.0	4.8	11	20	9
P 500, 3	103	63	32	10	0.3	0.4	2.8	10.0	6	10	4
P 500, 4	73	46	30	9	0.2	0.4	2.0	8.9	5	8	3
P 200, 1	39	26	15	3	0.1	0.4	4.3	2.8	9	13	4
P 200, 2	60	22	17	5	0.2	0.4	3.0	4.9	5	12	7
P 200, 3	28	23	17	4	0.1	0.2	2.7	4.3	5	6	1
P 200, 4	33	26	15	3	0.1	0.2	4.1	2.6	10	12	2

Table I-5: Comparison of oxygen demand before BOD with BOD and oxygen demand after BOD in 1st experiment

Name of the Sample	BOD	After BOD (COD)	Sum of BOD and after BOD (COD)	ThOD	Difference
P 1000,1	815	139	954	1003	49
P 1000,2	569	173	742	1003	261
P 1000,3	728	177	905	1003	98
P 1000,4	816	122	938	1003	65
P 500,1	298	82	380	623	243
P 500,2	410	101	511	623	112
P 500,3	472	103	575	623	48
P 500,4	393	73	466	623	157
P 200,1	100	39	139	141	2
P 200,2	84	60	144	141	-3
P 200,3	109	28	137	141	4
P 200,4	115	33	148	141	-7

Table I-6: Soluble Chemical Oxygen Demand (SCOD) and Total Chemical Oxygen Demand (TCOD) after BOD test

Pellet/ Sample	TCOD	SCOD
P 1000,1	197 ± 2	123 ± 0
P 1000,2	245.5 ± 9.5	129 ± 0
P 1000,3	252 ± 22	125 ± 0
P 1000,4	174 ± 0	126 ± 0
P 500, 1	117 ± 0	66 ± 0
P 500, 2	143 ± 0	73 ± 0
P 500, 3	147 ± 0	90 ± 0
P 500, 4	104 ± 0	65 ± 0
P 200, 1	97 ± 0	64 ± 0
P 200, 2	149 ± 0	55 ± 0
P 200, 3	69 ± 0	57 ± 0
P 200, 4	81 ± 0	64 ± 0

Table I-7: Comparison of Phosphate and Total Phosphorus before and after BOD test

Pellet/ Sample	PO_4 (After BOD)	TP (After BOD)	PO_4 (Before BOD)	TP (Before BOD)
P 1000,1	8.45 ± 0.15	9.35 ± 0.05	40.2 ± 0	56 ± 12
P 1000,2	7.5 ± 0.1	8.95 ± 0.05	40.2 ± 0	56 ± 12
P 1000,3	8.3 ± 0	10.65 ± 0.15	40.2 ± 0	56 ± 12
P 1000,4	8.9 ± 0	9.7 ± 0	40.2 ± 0	56 ± 12
P 500, 1	6.85 ± 0.05	7.8 ± 0.1	40.2 ± 0	56 ± 12
P 500, 2	6.6 ± 0	7.35	40.2 ± 0	56 ± 12
P 500, 3	8.15	8.95	40.2 ± 0	56 ± 12
P 500, 4	5.7	6.1	40.2 ± 0	56 ± 12
P 200, 1	4.35	5.25	40.2 ± 0	56 ± 12
P 200, 2	4.45	5.9	40.2	56
P 200, 3	4.3	5.95	40.2	56
P 200, 4	4.6	5.85	40.2	56
P 80, 1	3.65	5.05	40.2	56
P 80, 2	3.8	4.9	40.2	56
P 80, 3	3.6	4.9	40.2	56
P 80, 4	3.75	4.2	40.2	56

Table I-8: Comparison of Ammonia and Total Nitrogen before and after BOD test

Pellet/ Sample	NH_4 (After BOD)	TN (After BOD)	NH_4 (Before BOD)	TN (Before BOD)
P 1000,1	27.5 ± 0.5	32 ± 1	16.1 ± 0.8	24 ± 0
P 1000,2	22.5 ± 0.5	26 ± 0	16.1 ± 0.8	24 ± 0
P 1000,3	25.5 ± 0.5	35.5 ± 0.5	16.1 ± 0.8	24 ± 0
P 1000,4	31 ± 1	36.5 ± 0.5	16.1 ± 0.8	24 ± 0
P 500, 1	13 ± 0	17.5 ± 0.5	16.1 ± 0.8	24 ± 0
P 500, 2	18 ± 0	22 ± 1	16.1 ± 0.8	24 ± 0
P 500, 3	18.5 ± 0.5	23.5 ± 0.5	16.1 ± 0.8	24 ± 0
P 500, 4	14 ± 0	17 ± 0	16.1 ± 0.8	24 ± 0
P 200, 1	14 ± 0	16 ± 0	16.1 ± 0.8	24 ± 0
P 200, 2	13.5 ± 0.5	15 ± 1	16.1 ± 0.8	24 ± 0
P 200, 3	15 ± 0	17.5 ± 1.5	16.1 ± 0.8	24 ± 0
P 200, 4	14 ± 0	15.5 ± 1.5	16.1 ± 0.8	24 ± 0
P 80, 1	9 ± 0	12.5 ± 0.5	16.1 ± 0.8	24 ± 0
P 80, 2	10 ± 0	13.5 ± 0.5	16.1 ± 0.8	24 ± 0
P 80, 3	10 ± 0	13 ± 0	16.1 ± 0.8	24 ± 0
P 80, 4	9 ± 0	12 ± 0	16.1 ± 0.8	24 ± 0

Table I-9: Volatile Solid (VS), Total Fixed Solid (TFS) and Total Solid (TS) values after BOD test

Pellet/ Sample	TS	TFS	(TS*100/TFS)	VS	(TS*100/VS)
P 1000,1	37 ± 0	30 ± 0	80%	7 ± 0	20%
P 1000,2	38 ± 0	31 ± 0	81%	7 ± 0	19%
P 1000,3	47 ± 7	30 ± 0	65%	17 ± 7	35%
P 1000,4	38 ± 2	30 ± 0	79%	8 ± 2	21%
P 500, 1	37 ± 0	31 ± 0	82%	7 ± 0	18%
P 500, 2	38 ± 0	30 ± 0	81%	7 ± 0	19%
P 500, 3	45 ± 4	30 ± 0	68%	15 ± 4	32%
P 500, 4	43 ± 0	30 ± 0	70%	13 ± 0	30%
P 200, 1	37 ± 0	30 ± 0	81%	7 ± 0	19%
P 200, 2	42 ± 3	30 ± 0	71%	12 ± 2	29%
P 200, 3	41 ± 3	30 ± 0	74%	11 ± 3	26%
P 200, 4	37 ± 1	30 ± 0	82%	7 ± 1	18%
P 80, 1	42 ± 3	30 ± 0	71%	12 ± 3	29%
P 80, 2	42 ± 0	30 ± 0	72%	12 ± 0	28%
P 80, 3	56 ± 12	30 ± 0	54%	25 ± 12	46%
P 80, 4	43 ± 1	30 ± 0	69%	13 ± 1	31%

Table I-10: Volatile Suspended Solid (VSS), Fixed Suspended Solid (FSS) and Total Suspended Solid (TSS) values after BOD test

Pellet/ Sample	TSS	FSS	VSS	(TSS*100/VSS)
P 1000,1	0.69 ± 0.04	0.24 ± 0.06	0.46 ± 0.03	66%
P 1000,2	1.19 ± 0.04	0.66 ± 0.04	0.53 ± 0.00	44%
P 1000,3	0.60 ± 0.03	0.18 ± 0.02	0.42 ± 0.01	69%
P 1000,4	0.66 ± 0.06	0.30± 0.02	0.37 ± 0.04	55%
P 500, 1	0.48 ± 0.01	0.16± 0.02	0.32 ± 0.03	66%
P 500, 2	1.01 ± 0.02	0.57 ± 0.11	0.44 ± 0.09	43%
P 500, 3	0.52 ± 0.00	0.11 ± 0.10	0.40 ± 0.11	78%
P 500, 4	0.56 ± 0.04	0.30 ± 0.01	0.26 ± 0.02	46%
P 200, 1	0.92 ± 0.01	0.61 ± 0.02	0.31 ± 0.01	34%
P 200, 2	1.04 ± 0.03	0.67 ± 0.03	0.37 ± 0.06	36%
P 200, 3	0.43 ± 0.00	0.14 ± 0.04	0.30 ± 0.04	69%
P 200, 4	0.60 ± 0.02	0.32 ± 0.02	0.28 ± 0.00	46%
P 80, 1	1.00 ± 0.02	0.69 ± 0.04	0.32 ± 0.02	31%
P 80, 2	0.55 ± 0.00	0.24 ± 0.04	0.31 ± 0.04	56%
P 80, 3	0.52 ± 0.01	0.25 ± 0.02	0.27 ± 0.01	52%
P 80, 4	0.60 ± 0.04	0.30 ± 0.04	0.31 ± 0.00	50%

Appendix J: Results of chemical analysis of each pellet`s solutions and fish waste after BOD test 2

Table J-1: Total chemical oxygen demand values for each chamber after BOD test for P 200

		TCOD				
Chamber number	**Sample**	**COD Range (mg/L)**	**Value (mg/L) and dilution**	**Value in solution (mg/L)**	**Average (mg/L)**	**Overall Std. Error**
1	P200, P1	10-150	11 (1:10)	110	86.67	12
2	P200, P2	10-150	8 (1:10)	80		
3	P200, P3	10-150	7 (1:10)	70		
4	P200, Grinded Pellet 1	10-150	25 (1:10)	250	280	51
5	P200, Grinded Pellet 2	10-150	21 (1:10)	210		
6	P200, Grinded Pellet 3	10-150	38 (1:10)	380		
7	P200, Autoclaved grinded pellet 1	10-150	14 (1:10)	140	160	41
8	P200, Autoclaved grinded pellet 2	10-150	25 (1:10)	250		
9	P200, Autoclaved grinded pellet 3	10-150	9 (1:10)	90		

Table J-2: Results of chemical Analysis of each fish waste's solution after BOD test 2

Chemical analysis

Dates: 21,22 April 2018

Table..:Results of chemical Analysis of each fish watse's solution after BOD test

		TCOD						SCOD				PO4						TP						NH4						NO3						TN					
Chamber number	Sample	COD Range (mg/L)	Value (mg/L) and dilution	Value in solution (mg/L)	Average (mg/L)	Overall average (mg/L)	Std. Error	Value (mg/L) and dilution				PO4 Range (mg/L)	Value (mg/L) and dilution	Value in solution (mg/L)	Average (mg/L)	Overall average (mg/L)	Std. Error	TP Range (mg/L)	Value (mg/L) and dilution	Value in solution (mg/L)	Average (mg/L)	Overall average (mg/L)	Std. Error	NH4 Range (mg/L)	Value (mg/L) and dilution	Value in solution (mg/L)	Average (mg/L)	Overall average (mg/L)	Std. Error	NO3 Range (mg/L)	Value (mg/L) and dilution	Value in original solution (mg/L)	Average (mg/L)	Overall average (mg/L)	Std. Error	TN Range (mg/L)	Value (mg/L) and dilution	Value in solution (mg/L)	Average (mg/L)	Overall average (mg/L)	Std. Error
1	Fish waste 1	10-150	<10 (1:100)	-	-	1455	35	44 (1:10)	440	467.5	42	0.05-5	4.86 (1:10)	48.6	47.03	41.50	4	0.05-5	4.89 (1:10)	48.9	49.05	44.61	3	0.05-16	3.6 (1:10)	36	35.5	28.25	2	0.5-18	0.5 (1:10)	5	5	3.75	0	0.5-15	8.8 (1:10)	88	87.00	72.50	6
			>150 (1:10)	-	-			-	-				4.65 (1:10)	46.5					4.92 (1:10)	49.2					3.5 (1:10)	35					0.5 (1:10)	5					8.3 (1:10)	83			
													0.46 (1:100)	46																							0.9(1:100)	90			
2	Fish waste 2	10-150	16 (1:100)	1600	1445			59 (1:10)	590			0.05-5	4.44 (1:10)	44.4	43.60			0.05-5	5.15 (1:10)	51.5	48.75			0.05-16	2.6 (1:10)	26	25.5			0.5-18	0.5 (1:10)	5	4			0.5-15	7.8 (1:10)	78	76.67		
			129 (1:10)	1290									4.24 (1:10)	42.4					4.60 (1:10)	46					2.5 (1:10)	25					0.3 (1:10)	3					8.2 (1:10)	82			
													0.44 (1:100)	44																							0.7 (1:100)	70			
3	Fish waste 3	10-150	15 (1:100)	1500	1520			40 (1:10)	400			0.05-5	3.04 (1:10)	30.4	30.53			0.05-5	3.45 (1:10)	34.5	34.8			0.05-16	2.6 (1:10)	26	26			0.5-18	0.3 (1:10)	3	3			0.5-15	5.6 (1:10)	56	56.00		
			154 (1:10)	1540									3.02 (1:10)	30.2					3.51 (1:10)	35.1					2.6 (1:10)	26					<5(1:10)	-					5.2 (1:10)	52			
													0.31 (1:100)	31																							0.6 (1:100)	60			
4	Fish waste 4	10-150	14 (1:100)	1400	1400			44 (1:10)	440			0.05-5	4.48 (1:10)	44.8	44.83			0.05-5	4.50 (1:10)	45	45.85			0.05-16	2.6 (1:10)	26	26			0.5-18	0.3 (1:10)	3	3			0.5-15	7.1 (1:10)	71	70.33		
			>150 (1:10)	-									4.47 (1:10)	44.7					4.67 (1:10)	46.7					2.6 (1:10)	26					<5(1:10)	-					7.0 (1:10)	70			
													0.45 (1:100)	45																							0.7 (1:100)	70			

Appendix K: Results of Settling Velocity of fish pellets in sea water

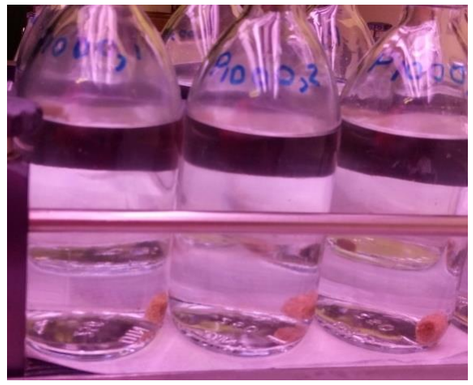
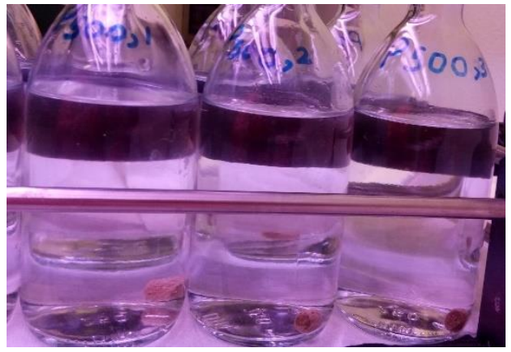
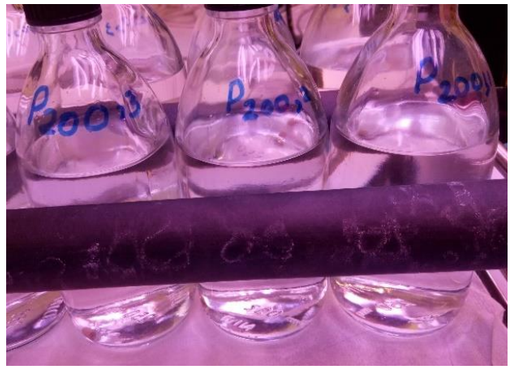
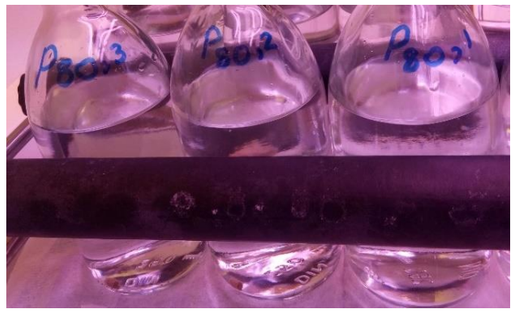
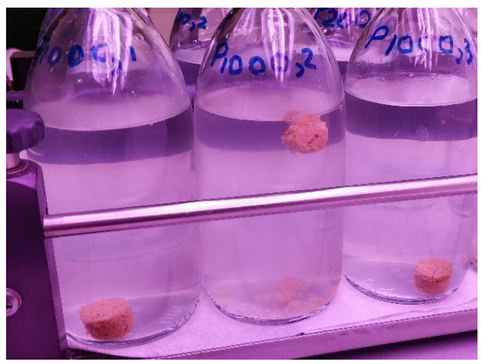
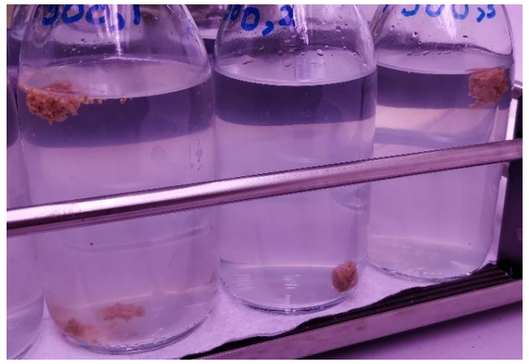
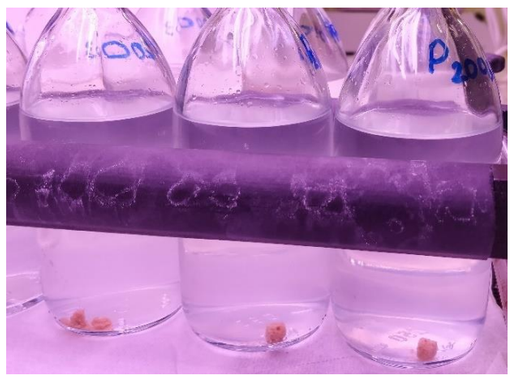
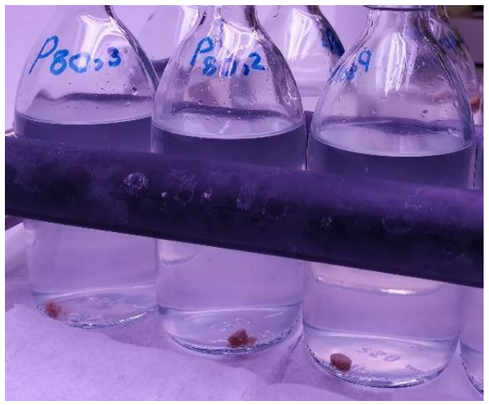
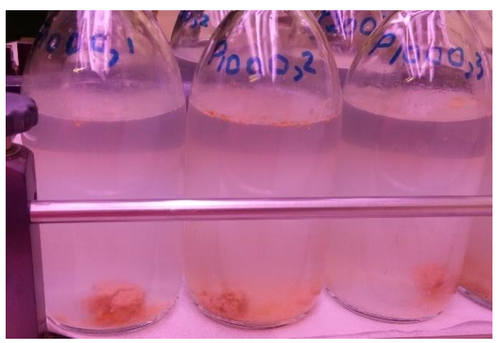
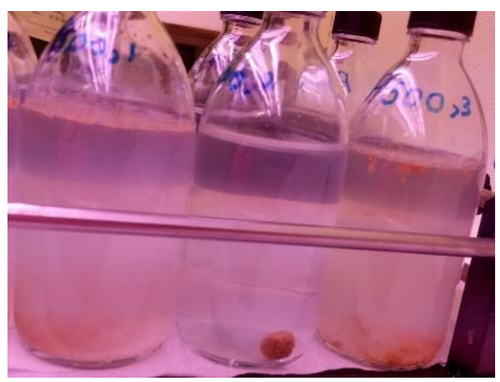
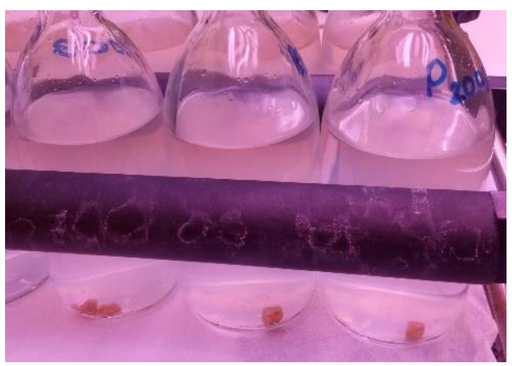
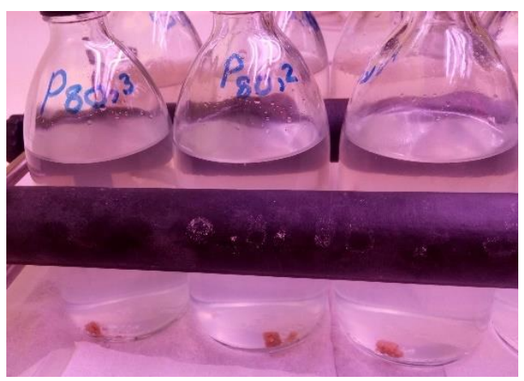
Table K-1: Settling Velocity of fish pellets in sea water

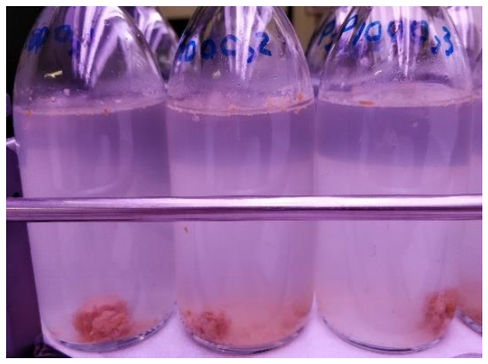
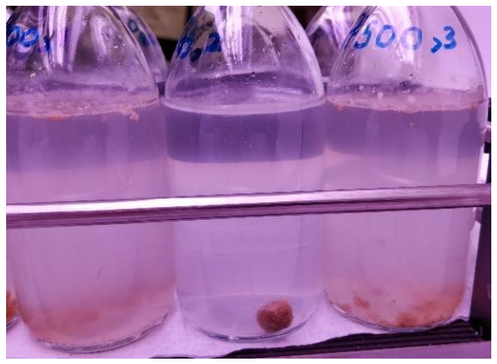
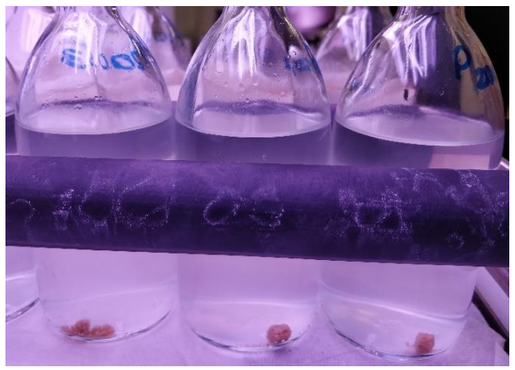
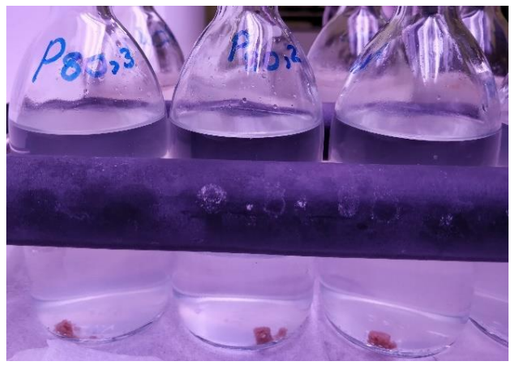
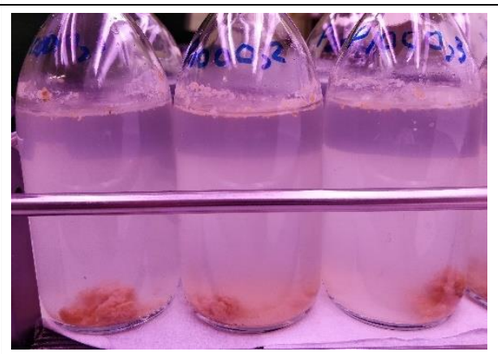
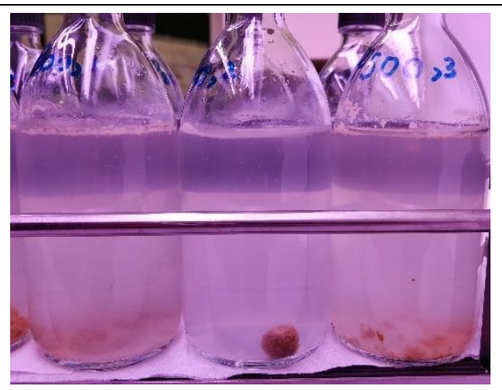
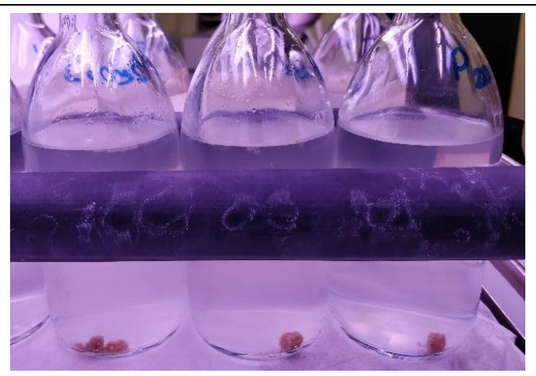
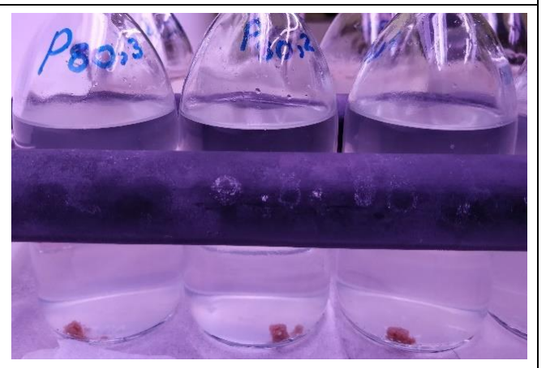
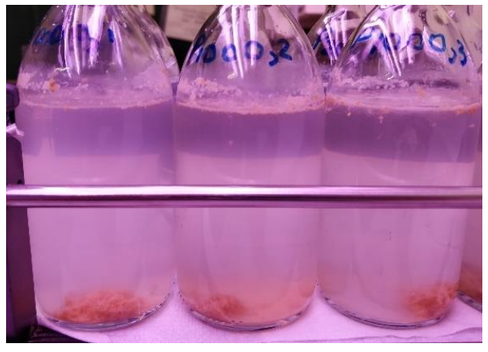
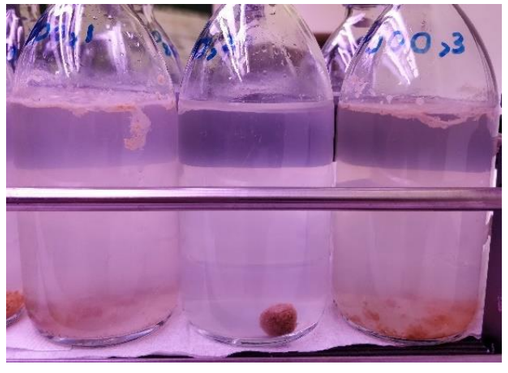
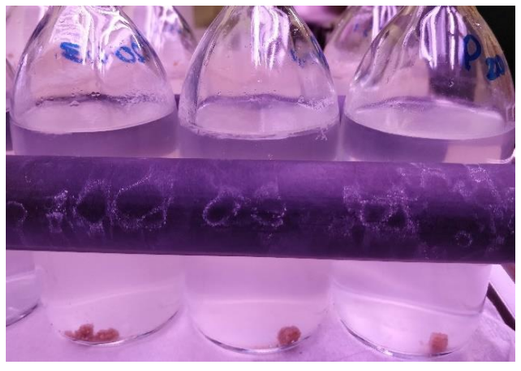
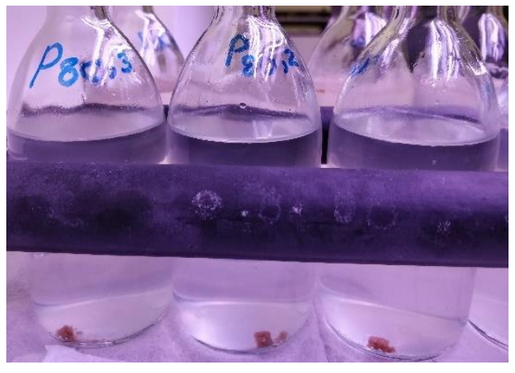
Hours	Time (s) - 1m	Time (s) - 10 cm	Velocity (1 m)- ms^{-1}	Velocity (10 cm) - ms^{-1}	Time (s) - 1m	Time (s) - 10 cm	Velocity (1 m)- ms^{-1}	Velocity (10 cm) - ms^{-1}	Time (s) - 1m	Time (s) - 10 cm	Velocity (1 m)- ms^{-1}	Velocity (10 cm) - ms^{-1}	Time (s) - 1m	Time (s) - 10 cm	Velocity (1 m)- ms^{-1}	Velocity (10 cm) - ms^{-1}
0	9	1.1	0.11	0.091	12	1.34	0.08	0.075	10	1.163	0.10	0.086	12.5	1.34	0.08	0.075
0.08	9.5	1.11	0.11	0.090	12	1.34	0.08	0.075	10	1.163	0.10	0.086	12.6	1.375	0.08	0.073
0.25	11	1.15	0.09	0.087	13	1.35	0.08	0.074	10.8	1.207	0.09	0.083	12.6	1.433	0.08	0.070
0.5	12	1.31	0.08	0.076	14	1.54	0.07	0.065	11.6	1.248	0.09	0.080	14.6	1.638	0.07	0.061
1	13	1.50	0.08	0.067	15	1.55	0.07	0.065	12	1.453	0.08	0.069	15.6	1.81	0.06	0.055
1.5	13.4	1.52	0.07	0.066	16	1.55	0.06	0.065	13.4	1.483	0.07	0.067	17	1.96	0.06	0.051
2	14	1.59	0.07	0.063	16.66	1.57	0.06	0.064	14	1.620	0.07	0.062	19.4	2.068	0.05	0.048
2.5	15.6	1.67	0.06	0.060	17	1.58	0.06	0.063	14.8	1.630	0.07	0.061	19.4	2.168	0.05	0.046
3	16	1.71	0.06	0.058	18	2.12	0.06	0.047	15	1.672	0.07	0.060	21	2.235	0.05	0.045
4	17	1.81	0.06	0.055	18	2.13	0.06	0.047	15	1.748	0.07	0.057	21	2.300	0.05	0.043
5	18	1.82	0.06	0.055	19	2.13	0.05	0.047	16	1.800	0.06	0.056	21.6	2.398	0.05	0.042
6	18.2	1.82	0.05	0.055	19	2.18	0.05	0.046	16	1.920	0.06	0.052	22.8	2.51	0.04	0.040
7	18.5	1.82	0.05	0.055	19	2.8	0.05	0.036	16.4	1.898	0.06	0.053	23	2.853	0.04	0.035
8	18.8	1.89	0.05	0.053	19	2.8	0.05	0.036	16.6	1.900	0.06	0.053	23	2.853	0.04	0.035
9	19	1.89	0.05	0.053	19	2.8	0.05	0.036	17	1.920	0.06	0.052	23	2.853	0.04	0.035

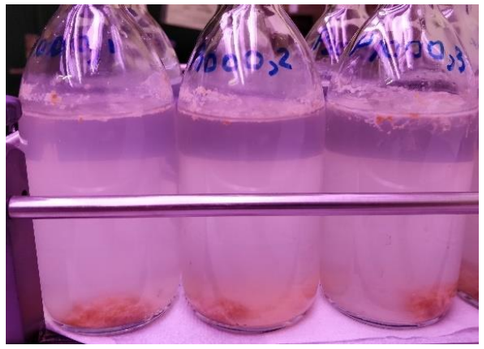
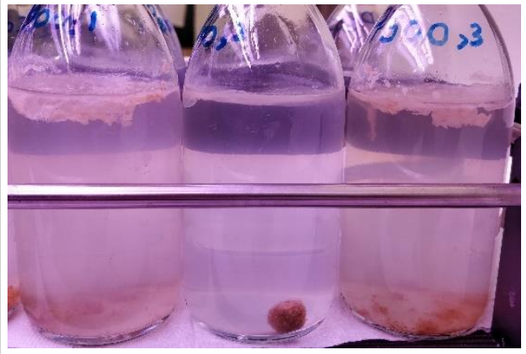
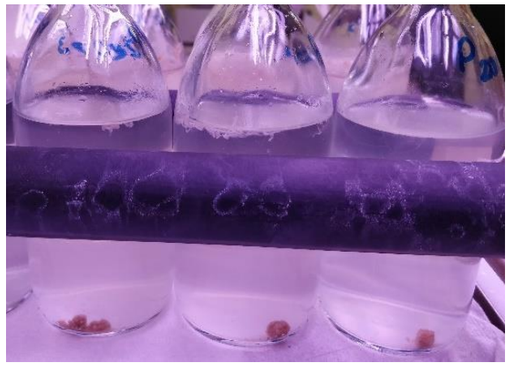
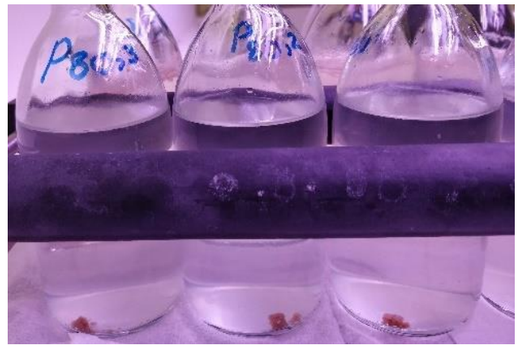
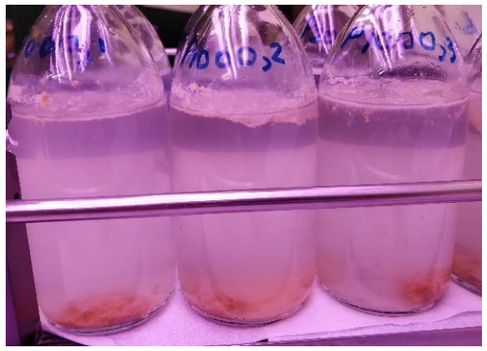
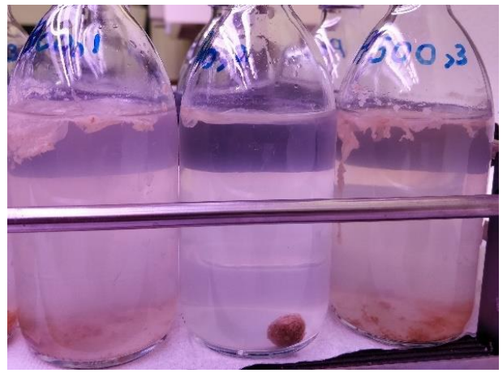
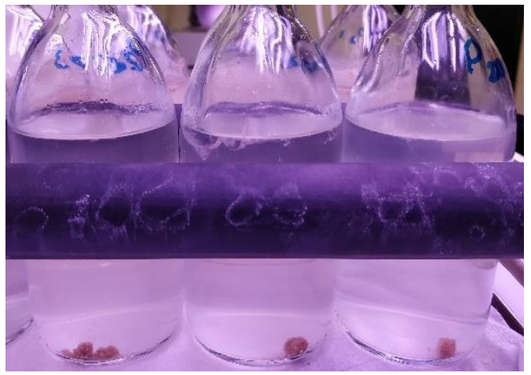
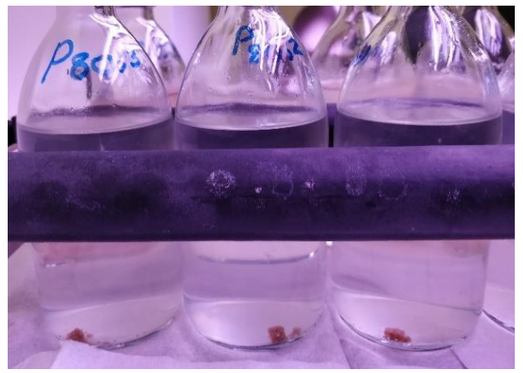
Table K-2: Volume and weight increment after 9 hours in a settling tube

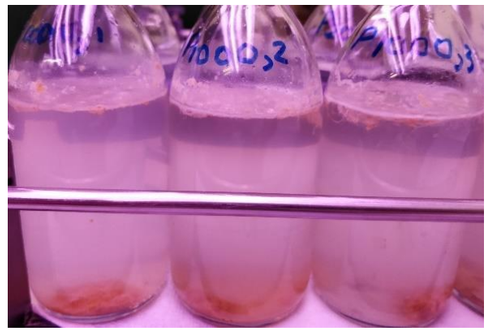
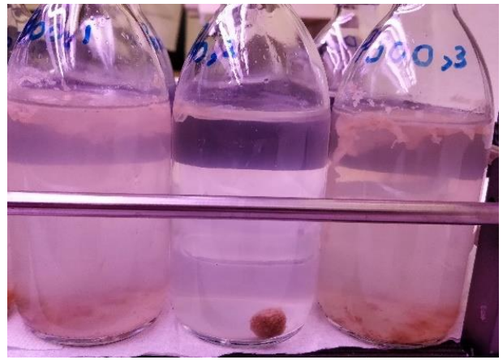
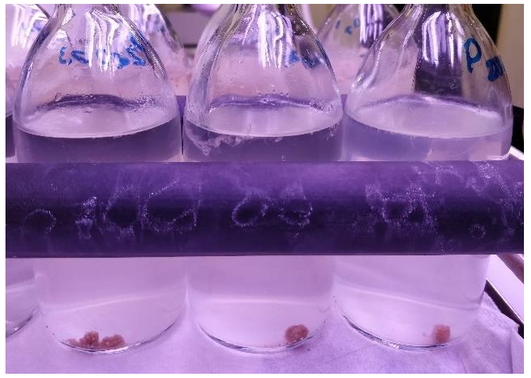
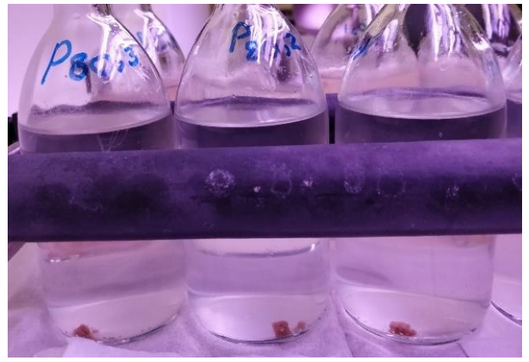
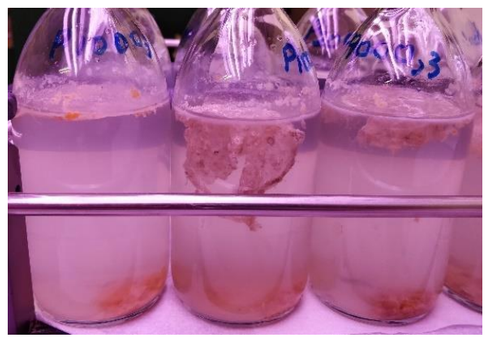
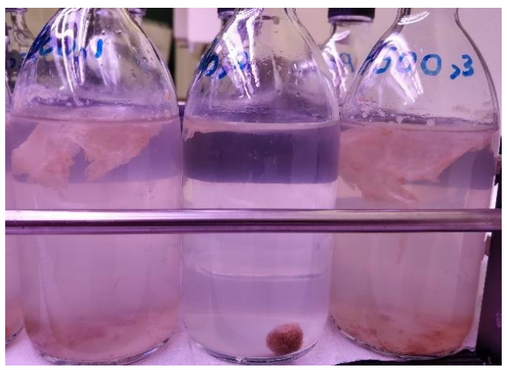
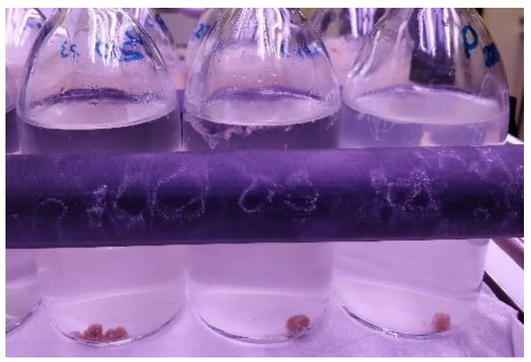
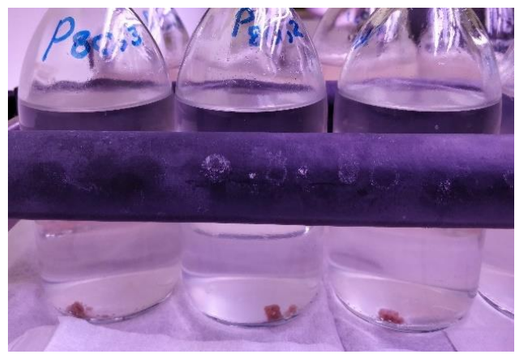
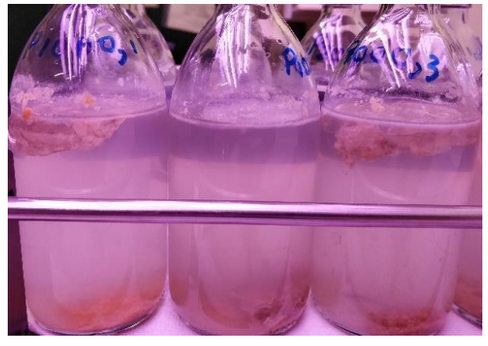
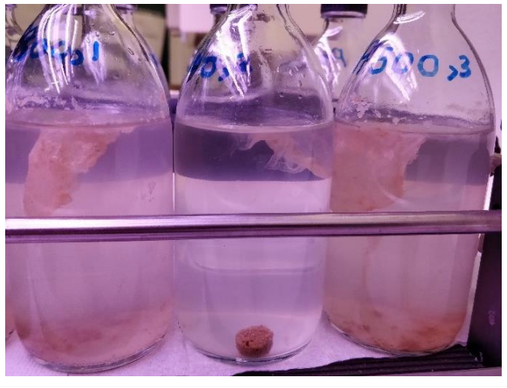
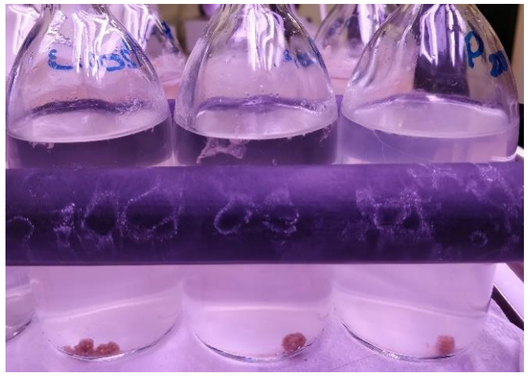
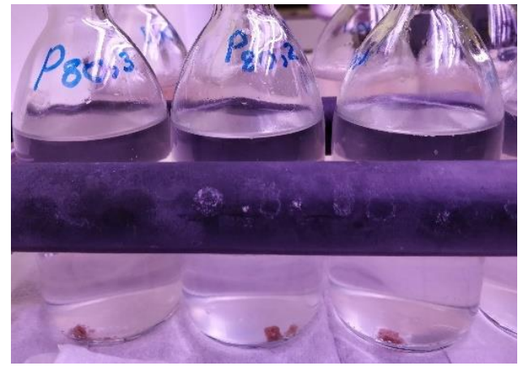
Size of Pellet	**Initial weight (g)**	**Final weight After 9 h (g)**	**Wt. increase (%)**	**Initial Volume (mm^3)**	**Final Volume After 9 h (mm^3)**	**Volume increase (%)**	**Color**	**Comments**
P1000	0.698	1.445	**106.81**	616	2155.1	**250**	Light brown	Double in size color fading, not a pellet (no hardness), fat layer
P 500	0.363	0.7007	**93.03**	313	863.9	**176**	Dark brown	Double in size, color fading, not a pellet (no hardness), fat droplets on the top
P 200	0.099	0.146	**47.47**	108	113.1	**5**	Dark brown	Round shape, No fat layer, particles visible, fade color, high speed
P 80	0.061	0.067	**9.84**	73	73.6	**1**	Dark brown	More round shape, no fat layer, particles there, swollen, fade color

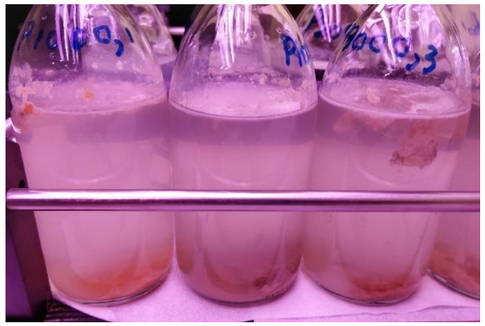
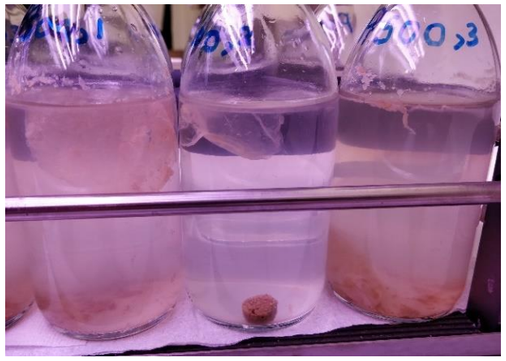
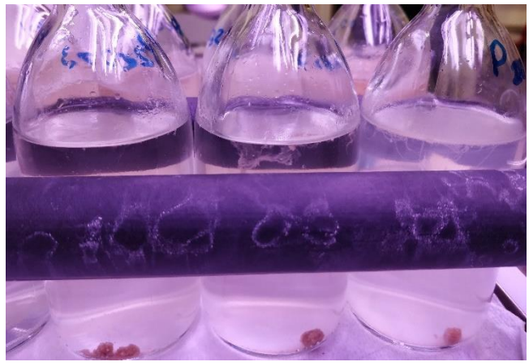
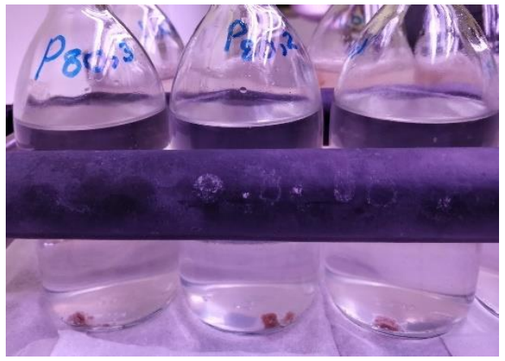
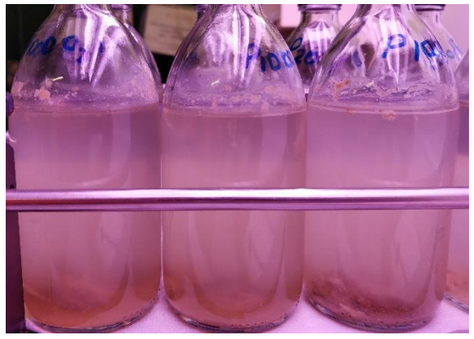
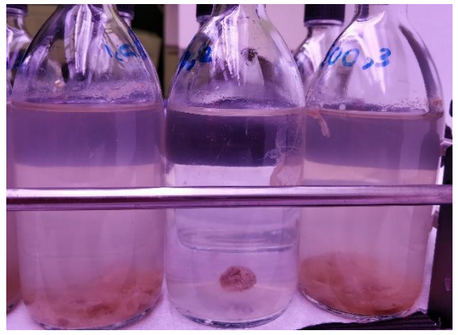
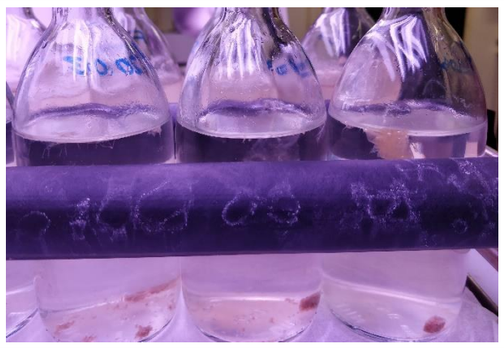
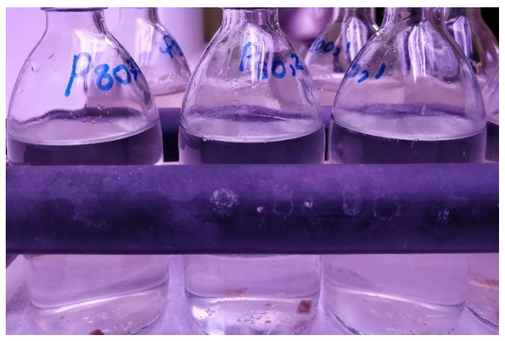
Appendix L: Disaggregation of fish feed pellets in sea water with time

	P 1000	**P 500**	**P 200**	**P 80**
Day 1				
Day 3				
	Double in size, some degrade small particle, many fat bubbles. One pellet is floating	Two pellets are Floating, some degrade small particle, some fat bubbles	Swollen pellet. One pellet is disaggregating into particles	Swollen pellet. Some are degraded particles can be seen
Day 5				

	Pellets further degrade into particles; fat bubbles appear as a layer on the top	Two pellets degrade into particles except middle one. fat bubbles appear as a layer on the top	Swollen pellet. One pellet is disaggregating into particles, no fat layer	Swollen pellet. Some are degraded particles can be seen, no fat layer
Day 7				
	Pellets further degrade into particles; fat bubbles appear as a layer on the top	Two pellets further degrade into particles except middle one. fat bubbles appear as a layer on the top	Pellets degrade slowly, and particles can be seen in the bottom	Pellets degrade slowly, and particles can be seen in the bottom
Day 9				
Day 13				

	Pellets further degrade into particles and appear as a collection of particles; fat bubbles appear as a layer on the top in whitish color.	Two pellets further degrade into particles except middle one and collection of particle start to spread, fat bubbles appear as a layer on the top in whitish color	Pellets degrade slowly, and particles can be seen in the bottom	Pellets degrade slowly, and particles can be seen in the bottom
Day 15				
Day 17				
	Pellets further degrade into particles and collection of particle spread in the bottom, fat bubbles appear as a layer on the top in whitish color	Two pellets further degrade into particles except middle one and collection of particles spread in the bottom, fat bubbles appear as a layer on the top in whitish color	One pellet in left hand side chamber disaggregate quickly than others	Pellets degrade slowly, and particles can be seen in the bottom

Day 20				
Day 22				
	Pellets further degrade into particles and collection of particle spread in the bottom, fat sheet is going to dip in the solution	Two pellets further degrade into particles except middle one and collection of particles spread in the bottom, whitish fat sheet is going to dip in the solution	One pellet in left hand side chamber disaggregate quickly than others	Pellets degrade slowly, and particles can be seen in the bottom
Day 24				
	Particles further spread in the solution, fat sheet is going further dip in the solution	Particles spread in the bottom except the pellet in middle chamber, whitish fat sheet is further dip in the solution	One pellet in left hand side chamber disaggregate quickly than others. Two pellets in right hand side still appear as pellet	Pellets degrade slowly, and particles can be seen in the bottom

Day 27				
	Particles further spread in the solution, fat sheet break and mix with solution	Particles spread in solution except the pellet in middle chamber, whitish fat sheet is further dip in the solution	One pellet in left hand side chamber disaggregate quickly than others. Two pellets in right hand side still appear as pellet	Pellets degrade slowly, and particles can be seen in the bottom
Day 45				
	Still particles and the fat layer can be seen. Fat layer started to degrade	Still particles and the fat layer can be seen. Fat layer started to degrade	Still particles remain in the solution, but not as pellet. Little fat layer is noticeable	Still particles remain in the solution, but not as pellet.