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Report

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Report Task 2.3: Particulate waste and turbidity in (marine) RAS

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Summary

Particulate waste management and removal is one of the most problematic parts of recirculation aquaculture systems (RAS). Particulate waste and thereby turbidity originates from three major sources: fish (faeces), feed and biofilm (heterotrophic bacteria and fungi). Based on size and density there are roughly four categories of particulate waste: settable, suspended, floatable and fine or dissolved solids. Specific problems related to high turbidity are a decreasing feed intake by fish, causing lower growth and increasing feed loss; an increasing risk regarding pathogens increasing disease problems and gill irritation; a higher oxygen consumption of the system and more complicate visual observation of fish, which is a necessity for good farm management.Good particulate matter and turbidity control is one of the key factors determining the success of RAS. The goal of this task is to get a better understanding of the nature of turbidity and the role of the different water treatment units within RAS and finally find ways to achieve a better control of turbidity.

The executed research is divided into four different parts; qualitative and quantitative analysis of suspended solids at critical points in RAS system; determine removal rates of the different system water treatment components; the relation of particle concentration and size distribution to turbidity and an evaluation of the use of flocculation as a practical method to manage turbidity.

A qualitative and quantitative analysis of suspended solids was made at critical points in the system by using the Coulter counter. Samples were taken from two systems; system B and system C/D; at different days during a period of one week. Sampling was done during feeding and in between feeding periods. Points of sampling within the systems were: Outlet grow-out unit (system B and C/D), outlet drum filter (system B) and outlet disk filter (system C/D), outlet trickling filter, outlet foam fractionator (system B) andoutlet up flow filter (system B). The size distribution and amount of particles are determined by using the Coulter counter. Sequential measurements of 1 ml in a series of 3 from small to larger particles (2-6 µm, 6-20 µm, 20-60 µm) were made. Removal rates of different system water treatment components were calculated by dividing the total amount of particles after and before the specific treatment modules.

To be able to evaluate the relation between the amount of particles, size distribution and turbidity, the particle and size distribution determined by using the coulter counter were related to turbidity levels determined by using the Hach DR 800 turbidity meter. Parameters were measured using a dilution range from 0 to 100% with steps of 10%. The original stock solution was diluted with filtered seawater. MilliQ water was used to keep salinity equal to the culture water used for the stock solution.

For the search of a practical method to manage turbidity a literature research regarding the use of flocculation was executed. Important questions as how flocculation works, which coagulant and or flocculant can or should be used, the time and place of addition of flocculants, the necessary dose and if flocculation is safe to use in aquaculture systems were treated. Besides literature research Jar tests were executed to evaluate efficiency of the available products. Toxicity tests were executed to estimate 24-hr LC_{50}, values for sole, which were compared to the functional dose. Finally the best combination of coagulant and flocculant were tested in a pilot system to evaluate optimal place of addition, removal rates of induced flakes using a drum filter, effect of hydraulic loading on efficiency of flocculation and safety.

Both in system B as system C/D the majority of the suspended solids were small particles. Both systems did show a high variation of suspended solids between the different days of sampling. Within system B the average amount of suspended solids between 2-6 um was approximately two times higher in between feeding than during feeding. The average amount of suspended solids in system B between 6-20um and 20-60um did not differ during feeding and in between feeding. Within system C/D the average amount of suspended solids between 2-6um, 6-20um and 20-60um did not differ during feeding and in between feeding.

The drum filter did clearly remove particles smaller than expected considering its screen size. The hypothesis that the smaller particles are attracted and "swallowed" by the large (feed) particles larger than 60 um, especially during feeding, and removed by the drum filter together seems plausible. In system B the drum filter, upflow filter and foam fractionator all clearly have a positive contribution towards removing particles during the period of sampling. The trickling filter did remove smaller particles, but produced larger particles. The disk filter also removed particles smaller than expected considering its screen size. The same hypothesis as mentioned with the drum filter seems likely. The trickling filter in system C/D did contribute to the removal of all particle sizes.

There is a relation of particle concentration and size distribution to turbidity, but the turbidity levels of the culture water tested were lying around the sensitivity levels of the Hach turbidity meter. From the calculated relation the trend is visible that turbidity is more related to small particles than larger ones. But more data is desirable to acknowledge this.

Flocculation is the use of coagulants and/or flocculants to agglomerate suspended impurities into larger filterable flakes, which than can be removed from the water by mechanical filtration. Primary coagulants are Iron or Aluminum salts and will neutralize the negatively charged suspended solids, eliminating electric repulsion, causing suspended solids to coagulate (micro flakes). Their efficiency and toxicity is pH dependent (both are high at low pH). Available products are Nicasal, Sachtoklar and Sachtoklar P. Secondary flocculants are polymers, such as polyacrylamide and potato starch, which use a combination of adsorption driven and electrostatic coagulation to increase size and density of the flock producing a macro flake. Their main function is to enhance the forming of larger and stronger flakes with better sedimentation characteristics. Available products are Synthofloc and Wisprofloc. Jar tests did show that the dose of a primary coagulant can be decreased with 33% in combination with a secondary flocculant. The optimal combination is Sachtoklar (P) with Synthofloc using a combined dose of respectively 0.164 g/l and 0.5 mg/l. The best time of addition is in between feeding periods when turbidity is highest and the primary coagulant needs to be added 10-60 seconds before the secondary flocculant. The 24-hr LC_{50} values are 1.30, 1.38 and 2,21 g/l for respectively Nicasal, Sachtoklar and Sachtoklar P. The functional dose of the primary coagulant is 8-14 times smaller than the LC_{50} values when used in combination with Synthofloc. The dose of Synthofloc is a factor 1000 smaller than the LC_{50} value. Wisprofloc is not toxic but 20 times more material is needed to achieve the same result. The combination of Sachtoklar P and anionic Synthofloc is the best choice considering efficiency, toxicity, organic loading and costs. Tests in a pilot system showed that the optimal place of addition of the primary coagulant is in the outlet of grow-out tank to create maximum contact time before the water goes into the mechanical filter. The place of addition of the flocculant is dependent of the place of addition of the coagulant and water flow. Flocculation can facilitate removal of (small) particles. The removal rates for induced flakes are 50-60% based on removal of dry matter content and 79% based on removal of Aluminum. The best option is to treat only 25% of the systems culture water through a by-pass, which gives advantages as a lower hydraulic loading and thereby a higher removal efficiency, a limited pH effect on the culture water and very important the Aluminum concentration did stay within safety limits so no acute toxicity risk is expected for Sole in grow out areas. But although flocculation seems to be an interesting practical method for managing turbidity further research is needed before this technology could be implemented

Introduction

Particulate waste management and removal is one of the most problematic parts of recirculation aquaculture systems (RAS) (Masser et al, 1999). Particulate waste and thereby turbidity originates from three major sources: fish (faeces), feed and biofilm (heterotrophic bacteria and fungi)(Franco Nava et al, 2004). Based on size and density there are roughly four categories of particulate waste: settable, suspended, floatable and fine or dissolved solids. Specific problems related to high turbidity are a decreasing feed intake by fish, causing lower growth and increasing feed loss; an increasing risk regarding pathogens increasing disease problems and gill irritation and a higher oxygen consumption of the system (Losordo et al, 1999; Losordo, et al 2000). High turbidity levels also complicate the visual observation of fish, which often is a necessary condition required for proper farm management. Therefore good particulate matter and turbidity control is one of the key factors determining the success of RAS (Malone and Beecher, 2000). The goal of this task is to get a better understanding of the nature of turbidity and the role of the different water treatment units within RAS and finally find ways to achieve a better control of turbidity. The executed research is divided into four different parts; qualitative and quantitative analysis of suspended solids at critical points in RAS system; determine removal rates of the different system water treatment components; the relation of particle concentration and size distribution to turbidity and an evaluation of the use of flocculation as a practical method to manage turbidity.

1. Qualitative and quantitative analysis of suspended solids at critical points in a RAS system

1.1 Description of suspended solids in a recirculation system for turbot at Zeeland Vis B.V. in the Netherlands

Zeeland Vis B.V. is a marine grow-out farm, situated in Yerseke at southwest of the Netherlands. The species cultured is turbot. The yearly production of 100 tons is realized in indoor recirculation systems, based on the latest developed technology. Construction of the facilities was finalized in spring 2001 and the farm is currently fully stocked and in full production. Zeeland Vis B.V. underlines that the research topics in this proposal all are very relevant for the turbot industry.

1.1.1 Experiments executed in March; system B and C/D in between and during feeding

Objective

Description of the amount and size distribution of suspended solids in system B and C/D to evaluate the contribution of the different water treatment modules installed within the systems.

Materials and methods

Samples of culture water were taken twice a day, during feeding and in between feeding, at three different days during a period of 7 days. During feeding represent the samples taken within the first 30 minutes after feeding the last grow-out unit. In between feeding represent the period 150 minutes after feeding the last grow-out unit. During a period of 7 days samples were taken from two different systems; B and C/D. System B does contain a drum filter, a trickling filter, an upflow filter and foam fractionator, while system C/D is simpler and does contain only a disk filter and a trickling filter. The flow schemes of both systems are depicted in figure S1 and S2. Both systems were monitored at their “critical” sampling points.

The points of sampling in system B:

1. Outlet grow-out unit (dark blue)
2. Outlet drumfilter, which is equal to the inlet of the trickling filter (pink)
3. Outlet trickling filter (green)
4. Outlet foam fractionator (light blue)
5. Outlet up flow filter (purple)

The points of sampling in system C/D:

1. Outlet grow-out unit (dark blue)
2. Outlet disk filter, which is equal to the inlet of the trickling filter (brown)
3. Outlet trickling filter (green)

The color mentioned between bars at each “critical” sampling point does correspond with the colors as shown in figure S1, S2 and the figures in the appendices. During the period of sampling water quality parameters, such as temperature, salinity, oxygen, pH, and suppletion, feeding rate and visual observations were recorded.

Measurement Coulter counter

The Coulter principle (Electrical Sensing Zone Method) used by the Coulter counter is the accepted "Reference Method" for particle size analysis and is the recommended limit test for particulate matter in large-volume parenteral solutions. The Coulter method of sizing and counting particles is based on measurable changes in electrical resistance produced by nonconductive particles suspended in an electrolyte. A small opening (aperture) between

electrodes is the sensing zone through which suspended particles pass (figure 1). In the sensing zone each particle displaces its own volume of electrolyte. Volume displaced is measured as a voltage pulse; the height of each pulse being proportional to the volume of the particle. The quantity of suspension drawn through the aperture is precisely controlled to allow the system to count and size particles for an exact reproducible volume. Several thousand particles per second are individually counted and sized with great accuracy. The method is independent of particle shape, color and density (Beckman Coulter, Inc 2006).

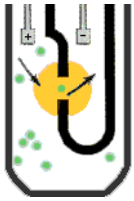

Figure 1: A graphic representation of the Coulter Counter principle.

Sequential measurements of 1 ml in series of three from small to larger particles; one sample: 1 ml three measurements 2-6, 1ml three measurements 6-20, 1 ml three measurements 20-60.

1. 2-6 µm
2. 6-20 µm
3. 20-60 µm

Depending on the concentration of the suspended solids in the culture water some samples needed to be diluted, with (filtered) water of the same salinity as the system water. No duplicate analysis was executed as it doubles analyzing time for mainly two reasons. Firstly duplicate analysis might create logistical problems when samples need to be diluted and secondly particles might agglomerate to larger ones due to adsorption while analyzing the first set of duplicates giving completely different results.

1.1.2 Experiments executed in June; system B during feeding

Objective

Description of the amount of suspended solids and size distribution of the particles in system B during feeding to get information about the contribution of the different water treatment modules installed within the system.

Materials and methods

Samples of culture water were taken once a day, during feeding at three different days during a period of 7 days. Samples were taken in an area without gas bubbles at the same depth of 0.5 m. The points of sampling in system B:

1. Outlet grow-out unit tray 1 (dark blue)
2. Inlet drumfilter, which is equal to the outlet grow-out tray 1-6 (brown)
3. Outlet drum, which is equal to the inlet of the trickling filter (pink)
4. Outlet trickling filter (green)
5. Outlet foam fractionator (light blue)
6. Outlet up flow filter (purple)

The flow scheme of system B is depicted in figure S1. The color mentioned between bars at each “critical” sampling point does correspond with the colors as shown in figure S1 and the figures in the appendices. During the sampling period water quality parameters, such as temperature, salinity, oxygen, pH, and suppletion, feeding rate and visual observations were recorded.

Measurement Coulter counter

Sequential measurements of 1 ml in a series of three from small to larger particles: As described in paragraph 1.1.1.

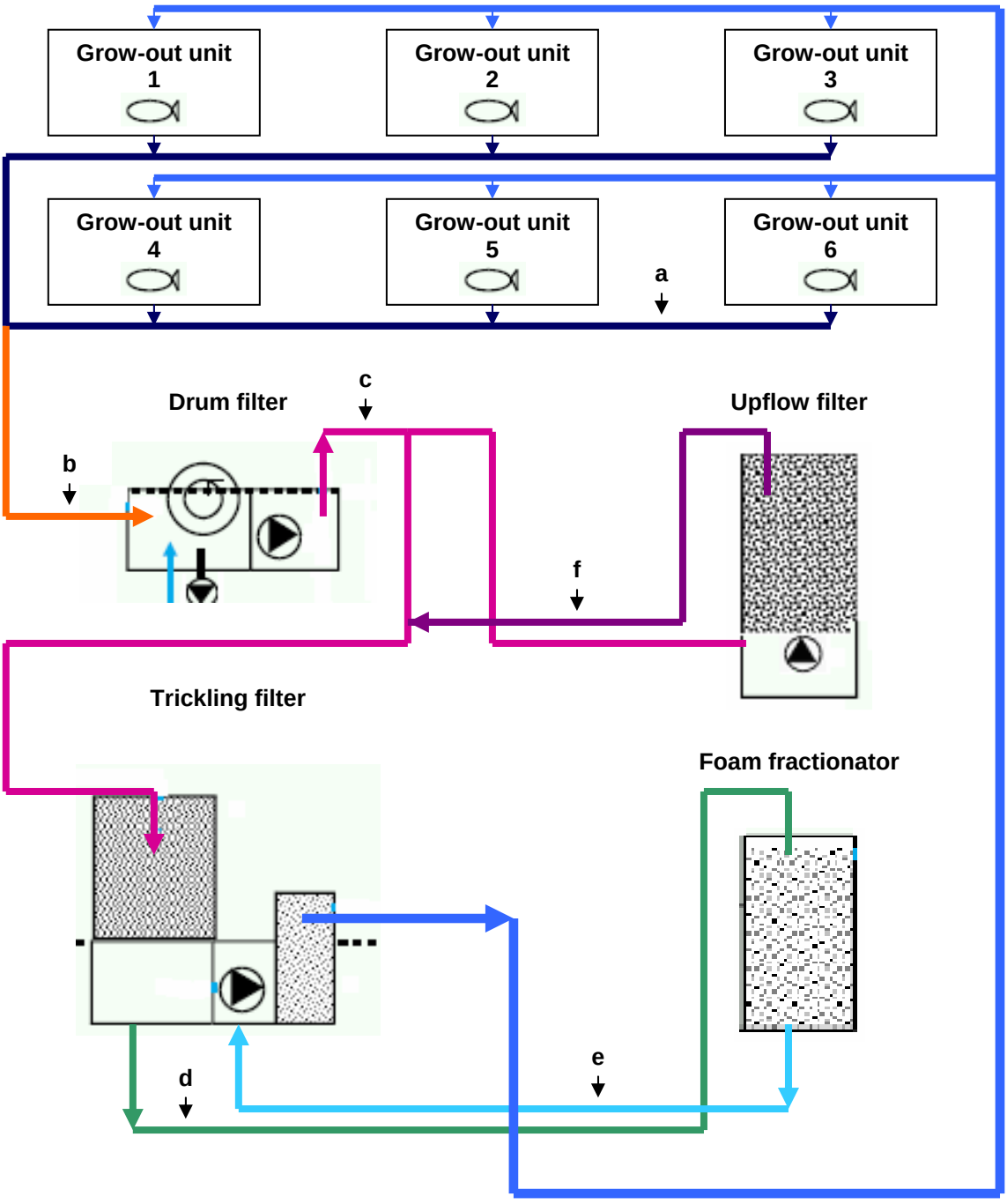

Figure S1: Flow scheme of system B. The different colors dark blue, orange, pink, green, light blue and purple correspond to the different sampling points in the following order; Outlet grow-out unit tray 1(a), Outlet grow-out unit tray 1-6 (b), Outlet drum filter (c), Outlet trickling filter (d), Outlet foam fractionator (e) and Outlet upflow filter (f).

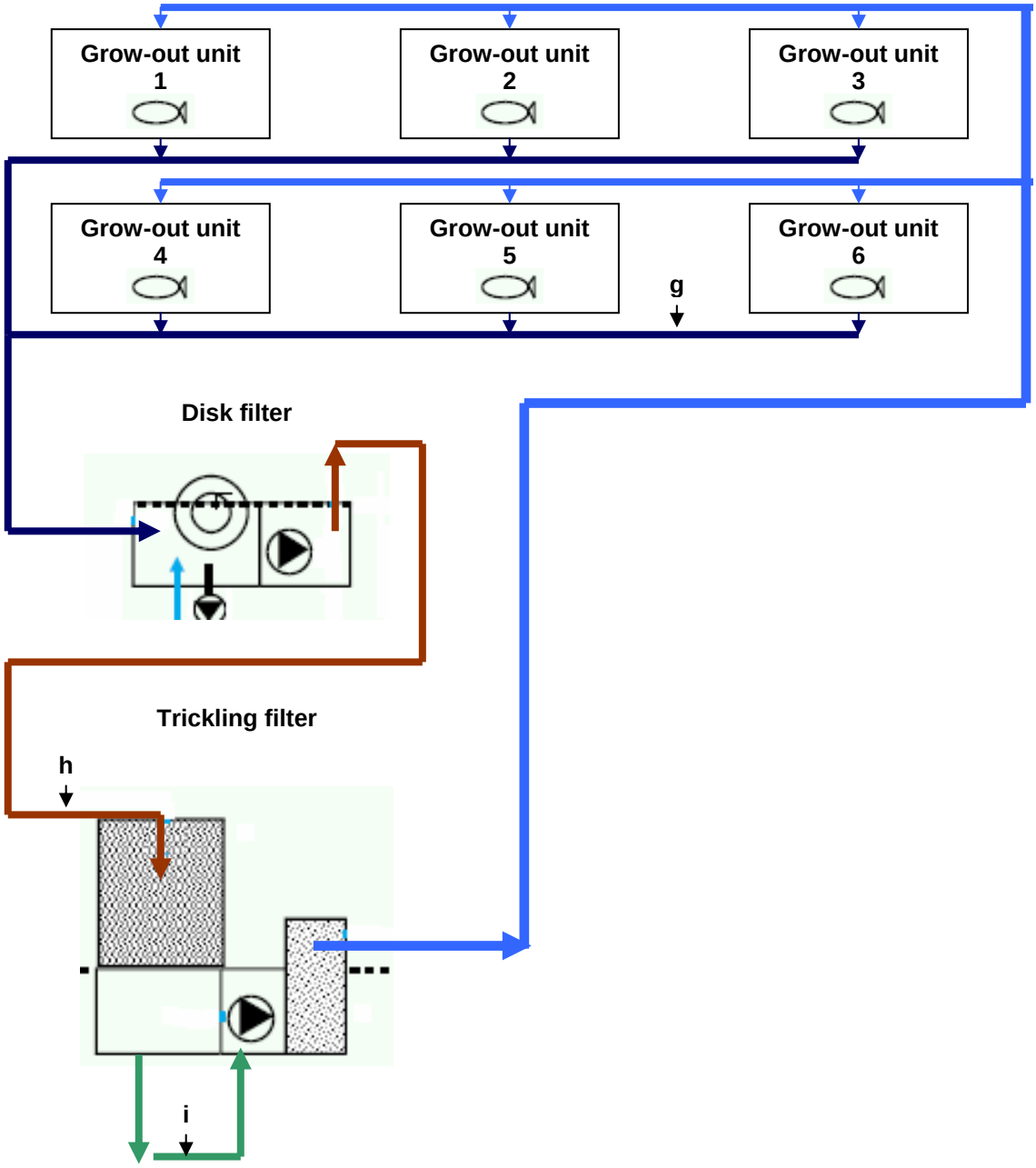

Figure S2: Flow scheme of system C/D. The different colors dark blue, brown, green and light blue correspond to the different sampling points in the following order; Outlet grow-out unit (g), Outlet disk filter (h) and Outlet trickling filter (i).

1.2 Results and discussion

1.2.1 Experiments executed in March; system B during feeding
As explained in the materials and methods data of suspended solids were determined in system B during feeding at 5 different sample points (outlet grow-out tray, outlet drumfilter, outlet trickling filter, outlet foam fractionator and outlet up-flow filter) and 3 different days in a period of 7 (1, 5 and 7). Results are shown in table L1, figure L1 to L13b and ANOVA AL1 to AL5 in appendix L. The line graphs are showing the size distribution at different sample points for different particle ranges at the specific day of sampling. The colors in the line graphs, which represent the data of the different points of sampling, comply with the colors of the flow scheme (figure S1). The column charts are showing the average amount of particles over the sample days at different sample points determined for the three different particle ranges; 2-6um, 6-20um and 20-60um. Water quality parameters varied from 17.9-18 ºC, 17 ppt, 6.7-6.8, 23.1% and 7.5-9.0 mg/l for respectively temperature, salinity, pH, suppletion, and oxygen. Feeding rate did not change during the period of sampling.

1.2.1.1 Suspended solids; particle range 2-6 um
The estimated amount of particles within the range of 2-6 um is affected by both the day of sampling (p=0.02) as point of sampling (p=0), after analyzing the data using a two-factor ANOVA without replication (AL3).

The effect of the different water treatment modules on the amount of particles is clearly visible in both the column chart (figure L10) as graphs L1, L4, L7 and L10b, which are showing the change of the size distribution for the suspended solids between 2-6 um at different sample points and day of sampling. The average of day 1, 5 and 7 at different sample points is shown in figure L10b. Looking at the calculated removal rates, which are all positive (table Lr1 and figure L 13b) it is clear that all modules did capture particles within 2-6 um at the time of sampling.

Most remarkable result is that although the screen size used in the drumfilter in system B is 40 um still an average of 33% of the small particles are removed. The hypothesis that during transport from the grow-out area to the drumfilter the smaller particles coagulate together or with larger particles in suspension forming particles large enough to be captured by the screen of the drum filter could be a plausible explanation.

Interesting is the pattern that small particles seem to be getting smaller further in the treatment process, which is noticeable in graph L1, L4, L7 and L10b by a shift to the left of the curves from the samples taken at points further into treatment process. An explanation could be that turbulence and pumping will cause some larger particles to break down into smaller ones.

It is important to realize when interpreting the data that the different water treatment modules are not all connected in series. The grow-out unit, the drumfilter and trickling filter are commentated in series, the upflow filter is connected parallel with the outlet basin of the drumfilter and the foam fractionator is connected parallel with the outlet of the trickling filter (Figure S1; scheme system B). Keeping system design in mind suspended solid data and removal rates of different modules can be compared. The results show that all treatment modules have a positive contribution to maintain water quality by removing particles within the range of 2-6 um at the time of sampling.

1.2.1.2 Suspended solids; particle range 6-20 um
The estimated amount of particles within the range of 6-20 um is affected by both the day of sampling (p=0.003) as point of sampling (p=0), after analyzing the data using a two-factor ANOVA without replication (AL4). The effect of the different water treatment modules on the amount of particles is clearly visible in both the column chart (figure L11) as in graphs L2, L5 and L11b, which are showing the change of the size distribution for the suspended solids between 6-20 um at different sample points and day of sampling. In figure L8 the amount of

particles is higher in the outlet of the drumfilter compared to the inlet, but the pattern looks fine. This result is unexpected and most likely caused by a faulty sample.

Looking at the calculated removal rates, which are all positive (table Lr1 and figure L 13) it is clear that all modules capture particles within 6-20 um at the time of sampling. It is a bit surprising though that the efficiency of the drum is higher for particles between 2-6 um than for particles between 6-20, but it partly underlines the hypothesis of coagulation regarding the smallest particles.

1.2.1.3 Suspended solids; particle range 20-60 um

The amount of particles determined within the range of 20-60 um is neither significantly affected by the day of sampling (p=0.78) as point of sampling (p=0.06; treatment module), although the effect of the different water treatment modules can still be visually detected, especially considering the calculated removal rates as shown in table Lr1 and figure L13b. But when looking at the size distribution determined within the range of 20-60 um (L3, L6, L9 and 12b) it is important to realize that the coulter counter always determines the amount of particles in a sample of 1 ml. It is logical and obvious that there can be put more small particles in 1 ml of water than larger ones. This is causing the data to be more scattered for the larger particles compared to the smaller particles.

Relying on the average removal rates the drumfilter, upflow filter and foam fractionator work as expected (capturing particles), but the trickling filter seems to produce particles between 20-60 um during the measurements. The latter is mainly due to the sample taken at day 1 (figure L3). But this finding suggests that the trickling filter sloughs of the larger particles formed from the material originating from the removal of the smaller sized particles with respectively 50% and 47% for 2-6 um and 6-20 um.

Table Lr1: Removal rates in of the different particle sizes per module in system B

Removal rates in %	Formula used	**Particle size**		
		2-6 um	**6-20 um**	**20-60 um**
Drum filter [1]	((Grow-out-drum)/grow-out)*100	33	4	50
Up flow filter	((Drum-upflow)/drum)*100	31	24	49
Trickling filter	((Drum-trickling)/drum)*100	53	47	-46
Foam fractionator	((Trickling-foam)/trickling)*100	54	24	20

[1] with SS tray 1 taken as value for SS grow-out tank.

1.2.1.4 Concluding remarks

- The drum filter, upflow filter, trickling filter and foam fractionator all capture suspended solids between 2-20 um at the time of sampling
- The drum filter did remove particles smaller than its own screen size at the time of sampling
- The majority of the suspended solids are small particles
- There is a high variation of suspended solids between different days of sampling
- The trickling filter did capture particles between 2-20 um, but did produce particles from 20-60 um at the time of sampling.

1.2.2 Experiments executed in March; system B in between feeding

As explained in the materials and methods, data of suspended solids were determined in system B in between two feeding periods at 5 different sample points; (outlet grow-out tray, outlet drumfilter, outlet trickling filter, outlet foam fractionator and outlet up-flow filter) and 3 different days in a period of 7 (1, 5 and 7). Results are shown in table Lr2, figure L14 to L26b and ANOVA AL6 to AL10 in appendix **L**. The line graphs are showing the size distribution at different sample points for different particle ranges at the day of sampling. The colors in the line graphs, which represent the data of the different points of sampling, comply with the colors of the flow scheme (figure S1). The column charts are showing the average amount of particles over the sample days at different sample points determined for the three particle ranges; 2-6um, 6-20um and 20-60um. Water quality parameters varied from 17.9-18 ºC, 17 ppt, 6.7-6.8, 23.1% and 7.5-9.0 mg/l for respectively temperature, salinity, pH, suppletion, and oxygen. Feeding rate did not change during the period of sampling.

1.2.2.1 Suspended solids; particle range 2-6 um

The estimated amount of particles within the range of 2-6 um is affected by both the day of sampling ($p=0.035$) as point of sampling ($p=0.001$), after analyzing the data using a two-factor ANOVA without replication (AL8). The effect of the different water treatment modules on the amount of particles is clearly visible in both the column chart (figure L23) as graphs L14, L17 L20 and L23b, which are showing the change of the size distribution for the suspended solids between 2-6 um at different sample points and day of sampling. The average of day 1, 5 and 7 at different sample points is shown in figure L23b. Looking at the calculated removal rates, which are all positive (table Lr2 and figure L26b) it is clear that all treatment modules did capture particles within 2-6 um at the time of sampling.

Just as found when sampling during feeding an average of 46% of the small particles are removed by the drumfilter even though the screen size used in system B is 40 um. Again the hypothesis that during transport from the grow-out area to the drumfilter the smaller particles coagulate together or with larger particles in suspension forming particles large enough to be captured by the screen of the drumfilter, seems likely. The pattern that small particles seem to be getting smaller further in the treatment process is also noticeable in graph L14, L17, L20 and L23b.

It is important to realize when interpreting the data that the different water treatment modules are not all connected in series. The grow-out unit, the drumfilter and trickling filter are commentated in series, the upflow filter is connected parallel with the outlet basis of the drumfilter and the foam fractionator is connected parallel with the outlet of the trickling filter (figure S1 scheme system B). Keeping system design in mind suspended solid data and removal rates of different modules can be compared. The results show that all treatment modules have a positive contribution to maintain water quality by removing particles within the range of 2-6 um at the time of sampling.

1.2.2.2 Suspended solids; particle range 6-20 um

The estimated amount of particles within the range of 6-20 um is neither affected by the day of sampling ($p=0.17$) as point of sampling ($p=0.12$), after analyzing the data using a two-factor ANOVA without replication (AL9). The effect of the different water treatment modules on the amount of particles is still visible in both the column chart (figure L24) as in graph L15 and L24b, which are showing the change of the size distribution for the suspended solids between 6-20 um at different sample points and day of sampling, but efficiency is minimal and variations are high, especially for the trickling filter and foam fractionator.

In figure L21 the amount of particles in the outlet of the trickling filter is higher than the inlet and the trickling filter seems to produce particles. The figure also gives the impression that the drumfilter is hardly working for particles between 6 and 20 um. Looking at the calculated removal rates (table Lr2 and figure L 26) it shows that the efficiency of the drumfilter to capture

particles within 6-20 um at the time of sampling is higher for particles between 2-6 um and 20-60 um than for particles between 6-20. This is complementary with the result found during feeding, which does support the hypothesis of coagulation regarding the smallest particles. With a negative removal rate of –14% the foam fractionator seems to produce particles during the time of sampling (L26b and table Lr2).

1.2.2.3 Suspended solids; particle range 20-60 um

The estimated amount of particles within the range of 20-60 um is affected by both the day of sampling (p=0.044) as point of sampling (p=0.004), after analyzing the data using a two-factor ANOVA without replication (AL10). Both the drumfilter as the upflow filter seems to capture particles, but the foam fractionator and especially the trickling filter are producing particles during the time of sampling (L25, L26b and table Lr2). These results support the suggestion made earlier with the data gathered during feeding that the trickling filter sloughs of larger particles, originating from the organic material captured due to the removal rate of respectively 58% and 47% for particles of 2-6um and 20-60um. But even though results are significant it is important to realize that the coulter counter always determines the amount of particles in a sample of 1 ml and that the amount of larger particles able to be determined are relatively small (AL10).

Table Lr2: Removal rates of the different particle sizes per module in system B

Removal rates in %	Formula used	**Particle size**		
		2-6 um	**6-20 um**	**20-60 um**
Drum filter [1]	((Grow-out-drum)/grow-out)*100	46	9	54
Up flow filter	((Drum-upflow)/drum)*100	51	28	10
Trickling filter	((Drum-trickling)/drum)*100	58	47	-100
Foam fractionator	((Trickling-foam)/trickling)*100	44	-22	-15

[1] with SS tray 1 taken as value for SS grow-out tank.

1.2.2.4 Concluding remarks

- The majority of the suspended solids are small particles
- There is a high variation of suspended solids between different days of sampling
- The average amount of suspended solids between 2-6 um is approximately two times higher in between feeding than during feeding
- The foam fractionator did produce larger particles during the time of sampling

1.2.3 Experiments executed in June; system B during feeding

As explained in the materials and methods data of suspended solids were determined in system B during feeding at 6 different sample points (outlet grow-out tray 1, outlet grow-out tray 1-6, outlet drumfilter, outlet trickling filter, outlet foam fractionator and outlet up-flow filter and 4 different days in a period of 6: (1, 2, 3 and 6). Results are shown in table T1, figure T1 to T32 and ANOVA AT1 to AT11 in appendix **T**. The line graphs are showing the size distribution at different sample points for different particle ranges at the day of sampling. The colors in the line graphs, which represent the data of the different points of sampling, comply with the colors of the flow scheme (figure S1). The column charts are showing the average amount of particles over the sample days at different sample points determined for the three particle ranges; 2-6um, 6-20um and 20-60um. Water quality parameters varied from 18.3-19.3 ºC, 17 ppt, 6.9-7.38, 11.1-12.1% and 7.7-10.1 mg/l for respectively temperature, salinity, pH, suppletion, and oxygen. Feeding rate did not change during the sampling period.

Striking is the difference regarding sample point 1 (grow-out tray 1) and 2 (grow-out tray 1-6). The amount of particles determined are higher for all particle ranges, while at first thought an equal concentration would be expected, assuming sample point 1 is a 6th part of the water at sample point 2 (refreshment rates are equal for every tank). But of course chances are small

feeding and fish metabolism are running synchronically for all tanks, as the tanks are not fed chronologically. Assuming an equal refreshment rate for all tanks, but feeding and faeces deposition is not synchronically sample point 2 can be treated as a blend of 6 samples (tray 1 to 6), which is expected to cause higher values of particles, but with a lower variation. The latter is clearly not the case and the cause seems unclear.

1.2.3.1 Suspended solids; particle range 2-6 um

The estimated amount of particles within the range of 2-6 um is neither affected by the day of sampling (p=0.20) as point of sampling (p=0.12), after analyzing the data using a two way ANOVA without replication. However when data from all sample days was pooled, suspended solids within the particle range 2-6 um are significantly different between the different water treatment modules (p=0.04; ANOVA single factor AT6).

Excluding the data from sample point 2 (grow-out tray 1-6) hardly affects the p value (0.045), but does result in a better visual presentation of the effect of the different water treatment modules on the amount of particles (figure T16). The same effect can be seen in the line graphs T1, T4, T8, T11 and T14b showing the change of the size distribution for the suspended solids between 2-6 um at different sample points and day of sampling and the calculated removal rates as shown in table Tr1 and figure T 32. Again it is remarkable to find that removal rates for smaller particles are higher than for the removal rates for larger particles, because the screen size of the drum is 40um.

1.2.3.2 Suspended solids; particle range 6-20 um

The day of sampling did not affect the amount of particles determined within the range of 6-20um (p=0.12). The amount of particles within the range of 6-20 is significantly different between sampling points (p=0.01) analyzing data using a two factor ANOVA without replication (AT3). When data from all sample days was pooled the difference found is even stronger (p=0; single factor ANOVA AT7), which is mainly due to the samples taken at sample point 2. After excluding the data from point 2 (grow-out tray 1-6) there is no significant effect (p=0.38) found, but the effect of the different water treatment modules can still be visually detected (T 18). The same effect is seen looking at the line graphs T2, T5, T9, T12 and T17b showing the change of the size distribution for the suspended solids between 6-20 um at different sample points and day of sampling. The calculated removal rates as shown in table Tr1 and figure T 32 acknowledge these results.

1.2.3.3 Suspended solids; particle range 20-60 um

The day of sampling did not affect the amount of particles determined within the range of 20-60 um (p=0.08). The amount of particles within the range of 6-20 is significantly different between sampling points (p=0.02) using a two factor ANOVA without replication. When data from all sample days was pooled the difference found is a little stronger (p=0.01; single factor ANOVA AT11), which is mainly due to the sample taken at point 2, day 6 (T13).

After excluding the data from sample point 2 (grow-out tray 1-6) there is no significant effect (p=0.19) found, but the effect of the different water treatment modules can still be visually detected (T31). The drumfilter, upflow filter and foam fractionator work as expected (capturing particles), but in this particular case the trickling filter seems to produce particles between 20-60 um during the measurements. The same effect is slightly seen looking at the line graphs (figure T3, T6, T10, T13 T20b and T31b) showing the change of the size distribution for the suspended solids between 20-60 um at different sample points and day of sampling. The calculated removal rates as shown in table Tr1 and figure T 32 acknowledge the results especially the negative removal rate regarding the trickling filter.

Table Tr1: Removal rates of the different particle sizes per module in system B

Removal rates in %	Formula used	**Particle size**		
		2-6 um	**6-20 um**	**20-60 um**
Drum filter [1]	((Grow-out-drum)/grow-out)*100	35	-10	26
Drum filter [2]	((Grow-out-drum)/grow-out)*100	73	67	52
Up flow filter	((Drum-upflow)/drum)*100	30	23	36
Trickling filter	((Drum-trickling)/drum)*100	18	15	-47
Foam fractionator	((Trickling-foam)/trickling)*100	34	25	17

[1] with SS tray 1 taken as value for SS grow-out tank; [2] With SS tray 1-6 taken as value for SS grow-out tank.

To compare all results it is important to realize that the coulter counter always determines the amount of particles in a sample of 1 ml. It is logical and obvious that there can be put more small particles than larger ones in 1 ml of water. This is causing the data to be more scattered for the larger particles compared to the smaller particles.

It is important to realize when interpreting the data that the different water treatment modules are not all connected in series. The grow-out unit, the drumfilter and trickling filter are commentated in series, the upflow filter is connected parallel with the outlet basis of the drumfilter and the foam fractionator is connected parallel with the outlet of the trickling filter (figure S1 scheme system B). Keeping system design in mind suspended solid data and removal rates are comparable and behave as expected.

1.2.3.4 Concluding remarks

- The majority of the suspended solids are small particles
- There is a high variation of suspended solids between different days of sampling
- The drum filter removes particles smaller than expected considering its screen size. The hypothesis is that the smaller particles are attracted and “swallowed” by the large (feed) particles larger than 60 um, especially during feeding, and removed by the drum filter together.
- The Trickling filter removes smaller particles, but does produce larger particles

1.2.4 Combined results system B

As can be seen from the figures L10b, T14b and L23b the majority of the suspended solids are small particles between 2-6 um.

The average total particles from 2-6 um of sample day 1, 5 and 7 are shown in figure P1 (Appendix P). These bars represent the integral of the different line graphs as shown in figure L10b for during feeding and L23b for in between feeding. As can be seen from the error bars in figure P1 there is a high variation of suspended solids between the different days of sampling, especially in between feeding periods. Within system B the average amount of suspended solids between 2-6 um is approximately two times higher in between feeding than during feeding (p=0). The average amount of suspended solids between 6-20um and 20-60um did not differ during feeding and in between feeding.

The average total particles from 20-60 um of sample day 1, 5 and 7 are shown in figure P2 (Appendix P). These bars represent the integral of the different line graphs as shown in figure L12b for during feeding and L25b for in between feeding. The average amount of suspended solids of 20-60 um did not differ during feeding and in between feeding (p=0.24) at the time of sampling. As can be seen from the error bars there is a high variation of suspended solids between the different days of sampling. Combining the removal rates of the system components during L13b and in between feeding L26b (figure P3 and P4) it can be seen that the trickling filter removes smaller particles, but did produce larger particles in both sample periods. Another interesting pattern can be noticed looking at the removal rates considering the foam fractionator. It seems that the foam fractionator contributes to the removal of all particle ranges measured during feeding, but often produces particles between 6-20um and 20-60um in

between feeding periods. As the amount of small particles are significantly higher in between feeding periods, the period the foam fractionator removes small particles, but produces larger particles it raises the question if its maximum removal capacity is reached at these moments. Also interesting is the pattern that the drum filter is showing higher removal capacities for particles between 2-6um than for 6-20 um. But this result appears to be highly dependent of the point of sampling, as can be seen in figure P5 and explained in 1.3.3.

1.2.5 Conclusions system B

- The majority of the suspended solids are small particles
- There is a high variation of suspended solids between different days of sampling
- The average amount of suspended solids between 2-6 um is approximately two times higher in between feeding than during feeding.
- The average amount of suspended solids between 6-20um and 20-60um do not differ during feeding and in between feeding
- The drum filter removes particles smaller than expected considering its screen size. The hypothesis that the smaller particles are attracted and "swallowed" by the large (feed) particles larger than 60 um, especially during feeding, and removed by the drum filter together seems plausible
- The drum filter, upflow filter and foam fractionator all clearly have a positive contribution towards removing particles.
- The Trickling filter removes smaller particles, but does produce larger particles
- In between feeding periods, when levels of small particles are higher than during feeding, the foam fractionator did capture small particles, but produced larger particles.

1.2.6 Experiments executed in March; system C/D during feeding

As explained in the materials and methods data of suspended solids were determined in system C/D during feeding at 3 different sample points (outlet grow-out tray, outlet disk filter and outlet trickling filter) and 3 different days in a period of 6 (1, 5 and 6). Results are shown in table Lr3, figure L27 to L39 and statistical sheet AL11 to LA15 in appendix L. The line graphs are showing the size distribution at different sample points for different particle ranges at the day of sampling. The colors in the line graphs, which represent the data of the different points of sampling, comply with the colors of the flow scheme (figure S2). The column charts are showing the average amount of particles over the sample days at different sample points determined for the three particle ranges; 2-6um, 6-20um and 20-60um. Water quality parameters varied from 16.3-16.7 °C, 23 ppt, 7.1, 23.1% and 8.6-9.2 mg/l for respectively temperature, salinity, pH, suppletion, and oxygen. Feeding rate did not change during the sampling period.

1.2.6.1 Suspended solids; particle range 2-6 um

The estimated amount of particles within the range of 2-6 um is affected by both the day of sampling ($p=0.01$) as point of sampling ($p=0.03$), after analyzing the data using a two-factor ANOVA without replication (AL13). The effect of the different water treatment modules on the amount of particles is visible in both the column chart (figure L36) as graphs L27, L30, L33 and L37b, which are showing the change of the size distribution for the suspended solids between 2-6 um at different sample points and day of sampling. Looking at the calculated removal rates, which are both positive (table Lr3 and figure L 39b) it is clear that both the disk filter as the trickling filter did capture particles within 2-6 um at the time of sampling.

Just as in system B it is found that although the screen size used in the disk filter is 40 um still an average of 24% of the small particles are removed. Again the most likely hypothesis is that during transport from the grow-out area to the disk filter the smaller particles coagulate together or with larger particles in suspension forming particles large enough to be captured by the screen of the disk filter.

In system C/D the different water treatment modules are connected in series (figure S2, scheme system C/D). Keeping system design in mind suspended solid data and removal rates of different modules can be compared. The results show that both the disk filter as the trickling filter have a positive contribution towards maintaining water quality by removing particles within the range of 2-6 um, with a removal rate of respectively 24% and 34%.

1.2.6.2 Suspended solids; particle range 6-20 um

The estimated amount of particles within the range of 6-20 um is neither affected by the day of sampling (p=0.74) as point of sampling (p=0.12), after analyzing the data using a two-factor ANOVA without replication (AL14).

As can be seen in figure L37 and concluded from the calculated average removal rate of 1% (table Lr3) the disk filter does hardly remove any particles within the range of 6-20 um. The limited effect of the disk filter on the amount of particles between 6-20 um at the day of sampling is also visible in figure L28, L31, L34 and L37b. At day 1 and 6 hardly any particles are removed while at day 5 the amount of particles in the outlet of the disk filter is even higher than compared to the amount of particles in the inlet. A disk filter normally does not produce particles, but it might be possible that larger particles in the disk filter are breaking down in (more) smaller ones, which are able to pass through the screen of the disk filter and thereby increasing the amount within the sample taken in the outlet.

With a calculated removal rate of 43% the trickling filter positively contributes to the removal of particles between 6 and 20 um. This effect is also shown in column graph L37 and figure L28, L31, L34 and L37b. After running a t-Test, between the results from the disk- and trickling filter assuming equal variances it appeared that the average amount of particles in the range 6-20 um are significantly (p=0.02, one tail) lower in the outlet of the trickling filter compared to the outlet of the disk filter (=inlet trickling filter).

1.2.6.3 Suspended solids; particle range 20-60 um

The amount of particles determined within the range of 20-60 um is not affected by the day of sampling (p=0.11), but does differ between the different sampling points (p=0.05) after analyzing the data using a two-factor ANOVA without replication (AL15).

Even tough figure L32 (day 5) show otherwise and variation of the data is high the removal capacity of the disk filter (59%, table Lr3) is solely responsible for this effect (figure L38 and L39B) as can be proven using a t-Test, which showed that the amount of particles between the inlet of the disk filter did differ from the outlet (p=0.02), but did not differ between the outlet from the disk and outlet from the trickling filter (p=0.46) (AL 15)..

Table Lr3: Removal rates of the different particle sizes per module in system C/D

Removal rates in %	Formula used	**Particle size**		
		2-6 um	**6-20 um**	**20-60 um**
Disk filter [1]	((grow-out-disk)/grow-out)*100	24	1	59
Trickling filter	((disk-trickling)/disk)*100	34	43	-3

[1] with SS tray 1 taken as value for SS grow-out tank.

1.2.6.4 Concluding remarks

- The majority of the suspended solids are small particles
- There is a high variation of suspended solids between different days of sampling
- The disk filter removes particles smaller than expected considering its screen size. The hypothesis is that the smaller particles are attracted and “swallowed” by the large (feed) particles larger than 60 um.
- The Trickling filter removes smaller particles, but does produce larger particles

1.2.7 Experiments executed in March; system C/D in between feeding

As explained in the materials and methods data of suspended solids were determined in system C/D in between feeding periods at 3 different sample points (outlet grow-out tray), outlet disk filter and outlet trickling filter and 3 different days in a period of 6 (1, 5 and 6). Results are shown in table Lr4, figure L39 to L52b and statistical sheet AL16 to AL20 in appendix **L**. The line graphs are showing the size distribution at different sample points for different particle ranges at the day of sampling. The colors in the line graphs, which represent the data of the different points of sampling, comply with the colors of the flow scheme (figure S2). The column charts are showing the average amount of particles over the sample days at different sample points determined for the three particle ranges; 2-6um, 6-20um and 20-60um. Water quality parameters varied from 16.3-16.7 ºC, 23 ppt, 7.1, 23.1% and 8.6-9.2 mg/l for respectively temperature, salinity, pH, suppletion, and oxygen. Feeding rate did not change during the sampling period.

1.2.7.1 Suspended solids; particle range 2-6 um

The amount of particles determined within the range of 2-6 um is not affected by the point of sampling (p=0.17), but differ between the different days of sampling (p=0.02) after analyzing the data using a two-factor ANOVA without replication (AL18).

The effect of the different water treatment modules on the amount of particles is slightly visible in the column chart (figure L49), but due to high variation and different patterns between the different sample days (figure L40, L43, L46 and L49b) the overall effect is not of significance. Patterns are as expected at day 5 and 6 (figure L43 and L46), but at the first day of sampling the trickling filter seem to produce particles (figure L40). Looking at the average removal rates, which are both positive (table Lr4 and figure L 52b) it is clear that both the disk filter as the trickling filter did capture particles within 2-6 um at the time of sampling.

In line with the other sample periods it is found that although the screen size used in the disk filter is 40 um still an average of 9% of the small particles are removed. As shown in figure S2 the different water treatment modules in system C/D are connected in series. The results show that both the disk filter as the trickling filter, but in this case especially the trickling filter has a positive, but modest contribution towards maintaining water quality by removing particles within the range of 2-6 um.

1.2.7.2 Suspended solids; particle range 6-20 um

The amount of particles determined within the range of 6-20 um is not affected by the day of sampling (p=0.21), but differ between the points of sampling (p=0) after analyzing the data using a two-factor ANOVA without replication (AL19).

Not expected, but clearly visible in figure L41, L44, L47, L 50 and L50b is that for all sample days in between feeding the amount of particles determined between 6-20 um in the outlet of disk filter is higher than the amount determined in the inlet (p=0.02; t-Test AL 19). The negative average removal rate of –17% for the disk filter regarding this range of particles acknowledges this (table Lr4, figure L52b). This result suggests that the disk filter actually does produce particles, which is unlikely. But as mentioned earlier, it might be possible that larger particles are broken down in (more) smaller ones due turbulence or hydraulic pressure forces in the disk filter. The smaller particles are able to pass through the screen of the disk filter and thereby increasing the amount within the sample taken in the outlet.

With an average removal rate of 35% (table Lr4, figure L52B) the trickling filter does capture a significant amount of the particles between 6-20 um (t-test; p=0.002 AL 19). The same pattern can be seen in figure L41, L44, L47 and L50b. On short term this will positively contribute towards maintaining water quality, but on long term the trickling filter will most likely clog. Clogging will create an anaerobic environment decreasing nitrification (NH3 removal) and attachment of biofilm to the medium's surface causing the biofilm to slough off. When the

biofilm sloughs off the biofilter produces high levels of particles, which will cause a quick deterioration of water quality as shown in the result obtained in system B.

1.2.7.3 Suspended solids; particle range 20-60 um

The estimated amount of particles within the range of 20-60 um is neither affected by the day of sampling (p=0.15) as point of sampling (p=0.40), after analyzing the data using a two-factor ANOVA without replication (AL20).

Even tough figure L42, L45, L48 and L51b together with the average removal rate in table Lr4 and figure 52b give the impression that the disk filter is producing particles and the trickling filter does capture particles, the effects are not significant even after running t-tests (AL20) comparing the amount of particles between in and outlet of the disk filter and trickling filter.

Table Lr4: Removal rates of the different particle sizes per modules in system C/D

Removal rates in %	Formula used	**Particle size**		
		2-6 um	**6-20 um**	**20-60 um**
Disk filter [1]	((grow-out-disk)/grow-out)*100	9	-17	-11
Trickling filter	((disk-trickling)/disk)*100	16	35	9

[1] with SS tray 1 taken as value for SS grow-out tank.

Looking at the overall effect it can be concluded that the trickling filter in system C/D has a higher contribution to removal of suspended solids than the disk filter, which is interesting to find.

1.2.7.4 Concluding remarks

- The majority of the suspended solids are small particles
- There is a high variation of suspended solids between different days of sampling
- Although not significant, looking at the removal rates the trickling filter did remove all particle sizes during the period of sampling.

1.2.8 Combined results system C/D

As can be seen from the figures L36b-L38b, and L49b-L51b the majority of the suspended solids are small particles between 2-6 um. The average total particles from 2-6 um and 20-60 um of sample day 1, 5 and 6 are shown in figure P6 and P7 (Appendix P). These bars represent the integral of the different line graphs as shown in figure L36b and L38b for during feeding and L49b and L51b for in between feeding. As can be seen from the error bars figure P6 and P7 there is a high variation of suspended solids between the different days of sampling. Within system CD the average amount of suspended solids between 2-60 um did not differ between feeding than during feeding. Combining the removal rates of the system components during L39 and in between feeding L52 (figure P8) it can be seen that the trickling filter does remove all the particle sizes during feeding as well as in between feeding periods. Surprisingly, the disk filter removes small particles in between feeding periods but produces larger particles in between feeding periods.
The only acceptable removal rates for the disk filter found do occur for the larger particles (20-60 um) during feeding periods. But this result is questionable as in system B it appeared that the removal efficiency of the mechanical filter is highly dependent of the point of sampling, as explained in 1.3.3**.** Unfortunately this finding cannot be acknowledged for system C/D as no sampled point comparable to grow-out tray 1-6 in system B is sampled in system C/D.

1.2.9 Conclusions system C/D

- The majority of the suspended solids are small particles
- There is a high variation of suspended solids between different days of sampling
- The average amount of suspended solids between 2-6um, 6-20um and 20-60um do not differ during feeding and in between feeding
- The disk filter removes particles smaller than expected considering its screen size. The hypothesis is that the smaller particles are attracted and “swallowed” by the large (feed) particles larger than 60 um.
- The trickling filter removes all particle sizes

1.2.10 Combined conclusions

It seems that the drumfilter works more efficiently than the disk filter although the removal efficiency of the mechanical filter is highly dependent of the point of sampling, as explained in 1.2.3.

2. Experimental set-up to relate particle concentration and size distribution to turbidity

2.1 Objective

Evaluate relation between coulter counter measurements and turbidity.

2.2 Material and methods

The particle concentration and size distribution of the suspended solids will be determined by using the coulter counter. Turbidity will be determined by using the Hach DR 800 as turbidity meter.

2.2.1 Sampling

At two specific days (day 1 and 2), a large sample (stock solution) of approximately 10 l will be taken from the system (B or D) with highest turbidity at that specific day. From this stock solution 10 individual samples will be taken for the preparation of dilutions. The stock solution (culture water) will be diluted, with (filtered) water of the same salinity as the system water. The seawater, which will be used to dilute the stock solution, is cleared from particles by filtration using 0.45 um mesh filter paper. Salinity will be adapted by using milliQ (0 ppt). Parameters will be measured using a dilution range from 0 to 100% with steps of 10%. From each diluted sample particle concentration and size distribution of the suspended solids, as turbidity will be determined. Measurements of dilutions will be done in duplicate.

2.2.2 Point and time of sampling

Point of sampling: before the drum filter.
Time of sampling: In between two feeding periods.

2.2.3 Measurement Coulter counter

Sequential measurements of 1 ml in a series of three from small to larger particles: One sample: 1 ml three measurements 2-6, 1ml three measurements 6-20, 1 ml three measurements 20-60.

- 2-6 µm
- 6-20 µm
- 20-60 µm

2.2.4 Measurement turbidity meter

Turbidity will be determined for every sample in duplicate by using the turbidity meter (Hach DR 800).

2.3 Results

The major problem encountered was that the turbidity levels of the culture water were lying around the sensitivity levels of Hach turbidity meter. The data is plotted in figure Y1, Y2 and Y3 in enclosure Y. The following relations were calculated:

Ø2-6 um) $y = 17806x + 16766$ ($R^2 = 0.73$)
Ø6-20 um) $y = 123.35x + 222.96$ ($R^2 = 0.67$)
Ø20-60 um) $y = 0.2635x + 8.984$ ($R^2 = 0.01$)

Although based on limited data it can be seen that turbidity is mainly influenced by particles between 2-20 um and hardly affected by particles between 20-60 um.

2.4 Conclusion

A trend is visible that turbidity is more related to small particles than larger ones. But more data is needed to validate the statement.

3. The use of flocculants, a practical method to manage turbidity?

3.1 Introduction

At Solea, the first and only sole farm in the Netherlands, it is observed that the grow-out of sole in the current recirculation system is not optimal. The hypothesis is that frequently occurring high turbidity levels of the culture water is a major cause of growth retardation, as it is well known that an increase of (fine) suspended solids can cause gill irritation and together with the dissolved organic solids can contribute significantly to the oxygen demand of the total system. The idea is that turbidity is mainly caused by (fine) suspended and dissolved solids, which are two of the four categories of the waste solids found in recirculation systems. Suspended solids will not settle and therefore cannot easily be removed in conventional settling basins. The most popular treatment for removal of suspended solids involves some form of mechanical filtration. Approximately 50 % of the total suspended solids in a recirculating system are fine suspended solids, which are solids smaller than 30 µm. Fine and dissolved solids cannot be economically removed by sedimentation or mechanical filtration technology. Foam fractionation or protein skimming is in contrary to mechanical filtration and sedimentation a reasonable successful technique in removing fine and dissolved solids from recirculating tank systems. Foam fractionation, as employed in aquaculture, is a process of introducing air bubbles at the bottom of a closed column of water that creates foam at the top air/water interface. As the bubbles rise through the water column, solid particles attach to the bubbles' surfaces, forming foam at the top of the column. The foam build-up is then channeled out of the fractionation unit to a waste collection tank. Although the efficiency of foam fractionation is subject to the chemical properties of the water, the process generally can be used to significantly reduce water turbidity and oxygen demand of culture systems. But, although efficient foam fractionation appears to be limiting for turbidity control in the marine recirculation systems specifically used for the grow-out of turbot and sole. The use of coagulants and flocculants to agglomerate the fine suspended solids together to form larger flakes, as proposed in the project, might be a possible practical solution to be able to solve the lack of turbidity control. In contrary to fine and dissolved solids the larger flakes can economically be removed form the system by using the already available mechanical filtration or sedimentation capacity. Therefore the use of flocculants to control turbidity of culture water in marine recirculation systems is experimentally tested in this project to evaluate its potential. Important questions as how does coagulation works, which coagulant and or flocculant can or should be used, the time and place of addition of flocculants, the necessary dose, efficiency and if flocculation is safe to use in aquaculture systems are evaluated.

3.2 How does it work; coagulation and flocculation, the use of flocculants

Often ultra-finely dispersed solids may still be present in water, even if the solution appears transparent. For some applications these solids must be removed from the water and mechanical filtration is not capable of cleaning such finely dispersed solids or suspended solids. These suspended impurities can be agglomerated by the use of coagulants or flocculants to form larger filterable flakes, which can be removed from the water by mechanical filtration. This process is referred to as flocculation. Purifying water by the use of flocculants is a technology, which is common practice in the process of producing drinking water. Most of the coagulants or primary flocculants used are salts of trivalent iron or aluminum, like $FeCl_3$, $Fe_2(SO_4)_3$ or $Al_2(SO_4)_3$. Bivalent salts can be used also, but their coagulative properties are not as good, which makes their use less efficient. After adding a primary flocculant to water the flocculate will irreversible hydrolysate, as shown in the formulas below, causing the pH of the water to increase.

1) $Al(H_2O)_6^{3+}$ + H_2O → $(Al(H_2O)_5OH))^{2+}$ + H_3O^+
2) $(Al(H_2O)_5OH)^{2+}$ + H_2O → $(Al(H_2O)_4(OH)_2)^+$ + H_3O^+
3) $(Al(H_2O)_4OH)^+$ + H_2O → $(Al(H_2O)_3(OH)_3)$ + H_3O^+
4) $(Al(H_2O)_3OH)$ + H_2O → $(Al(H_2O)_2(OH)_4)^-$ + H_3O^+

As can be seen in the first formula, $Al(H_2O)_6^{3+}$ is surrounded by six water molecules. In every step, during the process of hydrolysation, which contains out of four steps, a water molecule H_2O is exchanged by a OH^- causing the valence to decrease. The speed of hydrolysation and question if the process is going through all the four steps is highly dependent on the acidity (pH) of the water. At pH 5 only the first step of the reaction will take place leaving only $Al(H_2O)_6^3$ and $(Al(H_2O)_5OH))^{2+}$, which is toxic for the aquatic environment. At pH 8 the complete hydrolysation process will take place going through all the four steps leaving only $(Al(H_2O)_2(OH)_4)^-$. It is crucial that primary flocculants are quickly and homogeneously distributed over the medium to be treated, as only $Al(H_2O)_6^{3+}$ and $(Al(H_2O)_5OH))^{2+}$ are responsible for the electrostatic coagulation process, which causes the suspended solids to coagulated. The pH of the water has great influence on the speed of the hydrolysation process. The higher the pH the quicker the flocculant needs to be distributed to achieve an acceptable efficiency. Coagulation is achieved because $Al(H_2O)_6^{3+}$ and $(Al(H_2O)_5OH))^{2+}$ will neutralize the (in general) negatively charged suspended solids, eliminating the electrostatic repulsion, which is called destabilization. Due to their electric charge the finely dispersed suspended particles do not agglomerate to from larger flakes, but instead mutually repel each other staying in suspension. The partially or complete elimination of the electrical charge will reduce or eliminate the electrostatic repellent forces, which cause the Van der Waals forces between the suspended solids to become the dominant force causing the suspended solids to coagulate, from micro flakes and finally flocculate. A substance, which is solely specialized in destabilization, is called a coagulant or primary flocculant. After coagulation it is necessary that the suspended solids (micro flakes) flocculate to macro flakes, flakes large enough to be separated from the water by filtration or sedimentation.

Flocculation can be stimulated by adding a flocculant (flocculation aid) or sometimes even by increasing the concentration of the coagulant. Flocculation aids are polymers, which dependent on the charge of their ionizable groups, use a combination of adsorption driven and electrostatic coagulation to increase the size and density of the flock producing a macro flake and thereby the sedimentation and or filtration characteristics of the particles. Flocculation aids are only capable of coagulating suspended matter in water in combination with a primary flocculant or coagulant.
Anionic and non-ionic flocculants use adsorption as the major force causing coagulation cationic flocculants use a combination of adsorption driven and electrostatic coagulation to increase the size and density of the flock. Examples of often used flocculants are polyacrylamide and potato starch.

3.3 Available flocculants

3.3.1 Aluminum based flocculants/coagulants

The aluminum-based flocculants Sachtoklar, Sachtoklar P and Nicasal are all based on trivalent aluminum and very effective. The products all do contain approximately 5.3% of Aluminum salts and are frequently used to purify industrial wastewater and treatment of drinking water.

Sachtoklar contains the two-aluminum salts aluminum hydroxide chloride and aluminum hydroxide chloride sulfate and Sachtoklar P does contain only aluminum hydroxide chloride, but the products are either called poly aluminum chloride or PAC's. The general formula is defined as $Al_n(OH)_mCl_{(3n-m)}$. Sachtoklar and Sachtoklar P both comply with the EN 883 norm, which make them suitable for the purification of drinking water, but the compounds itself are irritable for (human) eyes and skin. Animal tests show a LC_{48}-0 concentration of 750 mg/l for goldfish and LD 50 of 5 g/kg for rats (oral administration).

Nicasal contains aluminum nitrate sulfate and is in contrary to the previous products mainly used in the paper industry. Nicasal is a relatively new product and does comply with the XXXVIth recommendation of the Bundesinstitut fur risicobewertung and toxicity information given for Sachtoklar and Sachtoklar P. The German based Sachtleben Chemicals Gmbh produces the aluminum-based flocculants described. As mentioned earlier iron based flocculates are also available, but even tough these products are cheaper its use for the application in aquaculture systems (sole and turbot) appears to have some disadvantages compared to the aluminum based flocculates. Flocks formed with the help of iron-based flocculates are much heavier, which at first sight seems to be an advantage because it will improve sedimentation. But in aquaculture systems it is preferred that the flocks stay in the water until they are trapped and separated from the water by the systems filtration unit as for example a mechanical (drum) filter. Besides the weight of the flakes formed the color also differs greatly. The use of aluminum salt is hardly noticeable while iron salt will make the water orange to brown in color.

3.3.2 Anionic and cationic flocculants

Synthetic polyelectrolytes such as the polymer polyacrylamide are high-molecular-weight substances with active, generally ionogenic, charged groups. These compounds support flocculation, the transition from micro- to macro-flakes, and improve flake characteristics such as size, density, and filterability and dewater ability. Acrylamide is an organic compound, which forms odorless white flakes. Acrylamide is stable at room temperature, but can quickly polymerize when brought into contact with oxidation compounds or ultra violet radiation. The general formula can be written as (C_3H_5NO). The polymerized form is non-toxic, but the monomer can cause peripheral neuropathy and certain forms of cancer in animals. There are signs that this is also the case in humans, but evidence is lacking. Acrylamide is highly soluble in water, neutral in pH and does not absorb in sediments, which are perfect characteristics to improve quick distribution over the water to be treated. Unused Acrylamide in water or sediment will be degraded microbiological within days. Chances of accumulation in fish are negligible. Polyacrylamide is widely used with the production of drinking water causing most humans to be exposed with the polymer on a daily base. As the monomer acrylamide does occur in several food products, especially fried products high in starch e.g. chips, the average daily intake for humans is high and estimated to be approximately 0.5 g/kg bodyweight a day. As a result of the diverse requirements in terms of charge, concentration and degree of polymerization, around 300 different products are available in the Synthofloc range from Sachtleben. In this project the choice is made to use only the anionic Synthofloc series 8022 is based on its lower toxicity (LC-50 for goldfish > 1 g/l compared to 0.75-12 mg/l) and higher efficiency compared to the cationic form.

Alternatives for the use of polyacrylamide are Wisprofloc N and P from AVEBE. These products are based on potato starch and therefore environmental and animal friendly. Wisprofloc N consist of purely potato starch while Wisprofloc P consists of modified potato starch. The general formula's for respectively Wisprofloc N and P are $(C_6H_{10}O_5)_n$ and $(C_6H_{15}OCl)_n$. Both products are used for the purification of drinking water and comply with the EN 1406 the European Standard for chemicals used for treatment of water intended for human consumption. Disadvantages of starch-based products are its high price and extra organic loading of the system, which can stimulate bacterial growth.

3.4 Jar tests using Aluminum salts

3.4.1 Introduction

To be able to go through all the available products and quickly evaluate the efficiency of a product simple jar tests were used. Jar tests are simple trail and error experiments in little jars, which makes it is possible to quickly evaluate the flake forming ability, dose-response relationship and reaction time needed in the specific medium, which in this case culture water. To be able to test and compare more than one product at the same time special jar-testers are

developed. These testers compare products under exactly the same circumstances like volume and mixing energy and directly measure the relative turbidity.

3.4.2 Material and Methods

In this project no special jar-tester was available and simple jars were used to test the available (primary) flocculant products. Jars were filled with 500 ml of culture water, obtained from the basin before the inlet of the drum filter. The specific (primary) flocculate was added and the solution was stirred for 10 seconds. After stirring the process of flocculation was evaluated visually regarding flake size using the categories –, -, + and ++ of which ++ is a very good result and – a bad result. The time needed to achieve proper flocculation was also recorded.

3.4.3 Results

No coagulation was achieved with any of the products tested at a concentration of 2 drops per liter. At a concentration of 4 drops per liter coagulation using Nicasal and Sachtoklar was good although the time need to achieve sufficient coagulation was more than 40 seconds. Sachtoklar P did show only minor coagulation, even after 60 seconds. At a concentration of 6 drops per liter all the products tested did show good results. Again Sachtoklar P did show the worst results. Nicasal did show the best results; this product achieved good to very good coagulation within 30 seconds. The results are summarized in table 1.

Table 1: Overview of results from the jar-tests

	2 Dr/l	**Duration**	**4 Dr/l**	**Duration**	**6 Dr/l**	**Duration**
Nicasal	–	-	+	40	++	26
Sachtoklar	–	-	+	48	++	33
Sachtoklar P	–	-	-	60	+/++	40

3.4.4 Evaluation of Aluminum salts using the coulter counter

3.4.4.1 Objective

Evaluation and acknowledgement of the visual observations made during the jar tests for aluminum salts

3.4.4.2 Material and method

Samples of culture water were taken before the drumfilter and put in a bucket. At first a filter (mesh size 60 um) was put down in the bucket and the water above the filter was used to determine the amount and particle size distribution of the culture water (control sample). Secondly the filter was removed and the primary coagulant was added to the same sample, while stirring for 10 seconds. 73 second after adding the primary coagulative the filter (60 um) is put in the bucket and again the water above the filter was used to determine amount and particle size distribution of the culture water after addition of the coagulative. Sequential measurements of 1 ml in a series of three from larger to small particles were executed: One sample: 1 ml three measurements 3-6, 1ml three measurements 6-20, 1 ml three measurements 20-60.

- 3-6 µm
- 6-20 µm
- 20-60 µm.

Concentrations of added coagulative were 2 and 6 drops per litre and equal to the concentrations used in the jar tests.

3.4.4.3 Results

Results of the coulter counter measurements are summarized in table **c1.** For interpretation of the data it is important to realize that all particles smaller than 3um (e.g. bacteria) are not determined in the control sample, but that some of these particles are included in the sample after addition of the coagulative as a part of the small particles are captured during the forming of flakes. Some of the particles smaller than 3 um will be a part of the small flakes formed and some particles of the larger flakes formed. The flakes formed between 3-60 um go through the 60 um filter and therefore will be part of the particles found by the coulter counter causing an increase in percentage of particles before and after addition of the coagulative, while flakes larger than 60 um, which are excluded from the samples are not included and can cause a decrease of the percentage. As a result addition of a coagulative does not always result in a decrease of the amount of particles. Particles between 6-20um are even increasing, especially when using Sachtoklar and Sachtoklar P in the concentration of 2 drops per liter.

Table c1: percentage of decrease or increase of the suspended solids as determined by the coulter counter.

Particle size	**Nicasal**		**Sachtoklar**		**Sachtoklar P**	
Concentration	*2 dr/l*	*6 dr/l*	*2 dr/l*	*6 dr/l*	*2 dr/l*	*6 dr/l*
3-6 um	-16	-49	+11	-16	+37	-11
6-20 um	+98	-69	231	+82	485	+14
20-60 um	-44	-07	-44	-50	+41	+08

Using care while interpreting the data it could be said that Nicasal when using 6 drops per liter does give the best results. These results correspond with the results in the jar test. For the other products the results of the jar tests were much more positive than the results suggested by data generated by the coulter counter. As shown in figure c1, c2 and c3 in Appendix F the amount of particles in the range of 6-20 um between the different flocculant products used can differ greatly.

3.4.4.4 Conclusion

Using care while interpreting the data it could be concluded that Nicasal at a dosage of 6 drops per liter does give the best results.

3.5 Jar tests with flocculants; Synthofloc, Wisprofloc

3.5.1 Introduction

In this experiment simple jars were used to test the efficiency of the flocculation aiding products Synthofloc (anionic and cationic) and Wisprofloc (N and P).

To be able to go through all the available products and quickly evaluate the efficiency of a product simple Jar tests were used. Jar tests are simple trail and error experiments in little jars, which makes it is possible to quickly evaluate the flake forming ability, dose-response relationship and reaction time needed in the specific medium, which in this case culture water. To be able to test and compare more than one product at the same time special jar-testers are developed. These testers compare products under exactly the same circumstances like volume and mixing energy and directly measure the relative turbidity.

3.5.2 Material and Methods

In this project simple jars were used to test the flocculation aiding capacity of the products Synthofloc (anionic and cationic) and Wisprofloc (N and P). The products were tested separately in combination with the different aluminum salts (primary coagulants). Jars were filled with 500 ml of culture water, obtained from the basin before the inlet of the drum filter. At first the flocculates were tested separately by varying the concentration. Secondly the flocculates were tested for their flocculating activity in combination with aluminum salts. In this test the optimal

concentration of aluminum salt, as found in the previous test, is combined with variable concentrations of flocculation aid products. When flocculation results were good the use of aluminum salt was decreased until achieved flocculation was decreasing. Evaluation was done visually regarding flake size and sedimentation characteristics.

3.5.3 Results

3.5.3.1 Synthofloc

No coagulation and or flocculation is achieved when using Synthofloc without a primary coagulant, even though in theory the cationic variant should be able to initiate flocculation through electrostatic coagulation.

In combination with aluminum salts the anionic version of Synthofloc showed far better results than the cationic version, especially in combination with Sachtoklar and Sachtoklar P. The results in combination with Nicasal were less promising. The concentration of Sachtoklar P could be decreased with 33% in combination with Synthofloc compared with its single use without negatively affecting flocculation. The optimal concentration of Synthofloc anionic 0.2% is 0.5 ml, which equals a concentration of 1 mg/l. This concentration is a factor 1000 lower than the LC50 level. For an optimal effect using the combination of Synthofloc and Sachtoklar P it is important to add the flocculant aid Synthofloc 10 seconds later after adding the primary flocculant Sachtoklar P. When using the combination instead of the aluminum salt only much larger flakes are formed, which also show much better sedimentation characteristics. Within minutes all suspended solids were trapped in flakes and laying as sediment on the bottom of the jar.

3.5.3.2 Wisprofloc

Both Wisprofloc N and P worked very well in combination with the aluminum salts Sachtoklar and Sachtoklar P. The results regarding flake size and sedimentation characteristics using the combination of Wisprofloc and aluminum salts were comparable with the results obtained with using Synthofloc, with the difference that 20 times the dry-matter content was needed using Wisprofloc compared to Synthofloc (0.4 ml of a 5% solution compared with a 0.5ml of a 0.2% solution). Just as noticed with Synthofloc it is necessary to add Wisprofloc 10 seconds later after adding the primary flocculant Sachtoklar P.

3.5.3.3 Conclusion

No coagulation and or flocculation occurred when using flocculants without a primary coagulant. With the exception of the cationic variant of Synthofloc all flocculant aids worked very good in combination with the aluminum salts Sachtoklar and Sachtoklar P. The concentration of Sachtoklar and Sachtoklar P and be decreased with 33% when using it in combination with a flocculant aid. The combined use of a coagulant and flocculant does result in large flakes, which reoccur quickly after induced fragmentation as for example caused by stirring. In jars the flakes will easily form sediment out of the water column.

3.6 Toxicity of Aluminum salts

3.6.1 Effects of aluminum on aquatic organisms

Flocculants based on aluminum are highly effective and generally give good results. But when using aluminum based flocculants for aquaculture ventures safety features are also of prime importance. Because the aluminum based primary flocculants are much more toxic than the flocculant aids as polyacrylamide only the Aluminum salts are treated.

The toxicity of aluminum is highly dependent on the acidity or pH of the water as is explained earlier in the figure depicting the hydrolysation formulas. At low pH (high acidity) the hydrolysation reaction is limited and almost all aluminum dissolved will be available as $Al(H_2O)_6^{3+}$ the most toxic form. It can actually be stated that the toxicity of aluminum is fully dependent en determined by the amount of $Al(H_2O)_6^{3+}$. At pH 5.2 the LC50 of aluminum for Atlantic salmon is only 51 ug/l. Problems are mostly related with ion-balance. At a neutral to high pH toxicity of aluminum is much less, LC50 values or NOEC's are unknown. Besides pH, dissolved fluorides and organic carbon can also influence the toxicity of aluminum. Organic carbon will produce flakes when aluminum is available and thereby capture aluminum by forming a shield causing toxicity to decrease. At high pH toxicity of aluminum is much less, but not completely safe. An often-occurring problem in fish, which was observed in this experiment as well, is the precipitation of aluminum polymers on the gill surface due to polymerization. To be able to evaluate the risk of using aluminum salts for sole in culture systems four values, respectively LC50 values, average exposed background concentration in the Netherlands and maximum allowable risk of exposure of total and dissolved amount of aluminum (MTR total and dissolved) are of importance. The average aluminum concentration in Dutch surface water is estimated to be approximately 100 ug/l. In the Netherlands the MTR (dissolved) and MTR (total) is estimated at respectively 312 ug/l and 1230 ug/l. It can be assumed that these values are taken on the safe side, as it is well known that water pH can differ greatly between different water bodies.

3.6.2 Estimating LC50's

As it appeared difficult to find toxicity values of aluminum salts for sole, it was decided to estimate the 24 hours LC50's for different available primary coagulates for sole during this project. The results obtained will be compared with the practical doses needed to make a evaluation for short term risks. No conclusion can be drawn about the effect of long term or chronicle exposure of aluminum salts for soles.

3.6.2.1 Material and methods

Exploratory experiments were executed to estimate practical and threshold concentrations for sole for each of the available primary coagulants. From the results obtained from the exploratory experiments the following sequence of concentrations tested for Nicasal, Sachtoklar and Sachtoklar P were respectively 0, 20, 30, 36, 42, 48 and 60 drops/l and 0, 40, 50, 60 and 70 drops per liter.

Tests were executed by using buckets filled with aerated culture water (1 l). Ten soles (7-10cm) were used per bucket. During the experiment ammonia, oxygen and pH were monitored. The experimental set-up is summarized in **table x:**

Table x: Experimental set-up for the estimation of LC50 values.

Concentration [Conc] in dr/l.	**Bucket 1 [Conc]**	**Bucket 2 [Conc]**	**Bucket 3 [Conc]**	**Bucket 4 [Conc]**	**Bucket 5 [Conc]**	**Bucket 6 [Conc]**	**Bucket 7 [Conc]**
Coagulant							
Nicasal	0	20	30	36	42	48	60
Sachtoklar	0	20	30	36	42	48	60
Sachtoklar P	0	40	50	60	70	-	-

3.6.2.2 Results and discussion

The estimated 24-hour LC50 values for Nicasal, Sachtoklar and Sachtoklar P were respectively 1,303; 1,380 and 2,213 g/l. The estimates were made using the data depicted in **figure To1 en To2** Appendix F**.** No mortality occurred at the buckets without added coagulants showing that the other parameters as oxygen, pH and ammonia were not limiting. Ammonia levels measured were low and oxygen levels varied between 5.8 and 8.6 mg/l. pH was influenced directly by adding the coagulants and especially at high concentrations pH values were low. During the experiment pH values rose in all buckets, which was quite remarkable. **Data is shown in table x2.**

Table X2: Oxygen levels (mg/l) and pH during the LC50's experiments.

Coagulant	**Concentration**	**O_2 after 1hr**	**O_2 after 24hr**	**Ph start**	*Ph end*	**PH rise**
Sachtoklar	0	5.8	7.5	7.77	8.09	0.32
	0.79	5.5	6.5	5.64	7.42	1.78
	1.19	6.1	6.9	5.03	6.90	1.87
	1.43	6.1	7.9	4.88	6.91	2.03
	1.67	6.0	8.3	4.84	5.47	0.63
	1.90	6.9	8.3	4.76	5.40	0.64
	2.38	6.3	8.2	4.68	5.16	0.48
Nicasal	0	7.5	8.0	7.65	7.94	0.29
	0.83	7.4	7.8	6.12	7.63	1.51
	1.24	7.3	8.2	5.13	6.42	1.29
	1.49	7.8	8.5	4.98	5.88	0.9
	1.74	7.0	8.6	4.88	5.38	0.5
Sachtoklar P	0	7.3	6.7	7.45	7.99	0.54
	1.61	7.1	6.6	5.64	7.06	1.42
	2.02	7.5	7.3	5.23	6.86	1.63
	2.42	7.4	8.2	5.04	5.85	0.81
	3.02	7.7	8.3	4.90	5.34	0.44

Using agents at equal concentration Sachtoklar P is compared to the other primary coagulatives tested the less toxic variant of the tested. This is most likely caused by the fact that Sachtoklar P in contrary to Sachtoklar does not contain aluminum-hydroxide-sulfate. The producer uses a LC0 value of 0.75g/l for goldfish. During previous experiments it appeared that when using a concentration of 6 drops per liter, no mortality occurred, even after a week.

3.6.2.3 Conclusion

The estimated 24-hour LC50 values for Nicasal, Sachtoklar and Sachtoklar P were respectively 1,303; 1,380 and 2,213 g/l, which does make Sachtoklar P the least toxic aluminum salt. These concentrations are 8 to 14 times higher than the concentration needed to clarify culture water in combination with flocculates (e.g. Synthofloc). It is important to realize that the real aluminum concentration the soles will be exposed to are lower than the concentration needed for flocculation as addition is done before the drum and a substantial part of the flakes, with aluminum enclosed, will be captured by the drum filter. Summarizing the results it can be concluded that the application of an aluminum salt as primary coagulant in combination with a flocculant seems to have no short-term risk for the stocked soles.

3.7 24 hours measurements

3.7.1 Introduction

The size-distribution and density of suspended solids in an aquaculture system follows a daily cycle. This cycle is mainly determined by the period of feeding, but can also be affected by the efficiency of the filters to remove suspended solids or the cycle of the biological filter itself to capture or produces particles (slough-off of biofilm material). Feeding does affect the amount of and distribution of suspended solids in two ways. Firstly by the addition of the feed itself and secondly addition of feed will stimulate metabolic activity, which will enhance production of faeces. The efficiency of the screen filters, and in this case the drum filter should be constant for the larger particles. The cycle of the biological filter, in this case a trickling filter is tricky. A biological filter can remove, but also produce suspended solids. It's cycle is dependent on many variables and difficult or impossible to predict, but in most cases not a daily cycle and most likely its efficiency of respectively removing or producing suspended solids will not change during the 24 hour measurement.

The efficiency of the flocculation process is determined by the amount and size of suspended solids in the culture water at the moment the flocculants are added. The highest efficiency is expected with a high concentration of suspended solids smaller than 40 microns, because it is expected that the drum filter, which has a mesh size of 40 um, will automatically remove larger particles. To optimize the process of flocculation it is crucial to add the flocculant at the moment suspended solids concentration is maximal. To estimate the time between feeding and maximum suspended solid concentration a 24-hour measurement is crucial to determine the cycle of suspended solids in the system.

3.7.2 Material and Methods

It was decided to measure every three hours during a period of 24 hours on three different spots; before the drum filter, after the drum filter and after the trickling filter. This makes it possible to estimate the efficiency of the drum and trickling filter together with the estimate of the cycle regarding density and size distribution in the system due to feeding. It is assumed that the efficiency of the screen filter, in this case a drum filter, should be constant for the larger particles and can be determined. It is also assumed that the cycle of the trickling filter is not a daily cycle and therefore its "efficiency" of respectively removing or producing suspended solids can also be determined. The sample procedure consists of the following steps:

1) Sampling by using a bucket before and after the drumfilter and after the trickling filter
2) A part of the sample will be filtered to prevent clothing of the coulter counter using a screen filter with mesh size of 100 um.
3) Two cuvettes are filled with the filtered water to estimate the size distribution and density by using the coulter counter.
4) Out of the unfiltered water two samples (duplo) are taken to determine dry matter content.
5) Coulter counter measurements are executed it three ranges; 3-6 um, 6-20um and 20-60um in duplicate, starting with the last sample to prevent sedimentation to be able to influence the measurements.

To support the interpretation and evaluation of the coulter counter measurements dry matter content and Secchi-disk values to represent turbidity are determined before and after the drum filter and trickling filter. Turbidity estimates by using the Secchi-disk could only be executed before and after the drum filter. Estimates after the trickling filter are not possible.

3.7.3 Results and discussion

The results obtained are shown in figure y1 and figure y2 in Appendix F. In figure y1 the amount of suspended solids are depicted against a daily 24-hour period. The little bar chart on top of figure y1 corresponds with the x-axis and does represent the feeding times. The height of the bar chart does represent the amount of feed fed at that specific feeding time. It seems there is no clear cycle between feeding time and amount of suspended solids, although the increase of suspended solids between midnight and 09:00 is most likely caused by the higher feeding rates given between 18:00 hours and midnight. As shown in figure y2 particles between 6-20 um are primarily responsible for the increase of the total amount of suspended solids. At the same time the amount of particles between 3 and 6 um are decreasing, which at this time can't be explained.

The amount, in number of particles between 20-60 um is neglect able compared to the smaller particles, but they represent 15% of the total dry-matter content. This is easily explained by the method of analysis using the coulter counter. The coulter counter does use 1 ml to analyze the amount of particles. It is obvious that the maximum amount of particles that can occur in 1 ml of water will decrease sharply as particles increase in size.

The amount of particles between 6 and 20 um found before the drum, before (is after the drum) and after the trickling filter were about the same and all rose in time. This shows that the trickling filter did not release particles and that the fish in the raceways also did not directly produce particles.

In figure y3 appendix F dry-matter content of the culture water before the drum filter together with feeding time and feeding rates are shown during a 24-hour period. Contrary to the previous data dry-matter content is related to feeding. Dry matter content will rise as soon as feeding takes place. Feed particles clearly affect dry matter content of the samples.

The Secchi disk values shown in figure y4 appendix F follow this pattern as well, but rising Secchi disk values do represent an increasing transparency or lower turbidity, while dry-matter content does clearly increase. This sounds contradictory, but transparency or turbidity is mainly influenced by the amount of smaller particles, while feed particles are often the larger, settable particles or solids.

But if turbidity is mainly influenced by the amount of smaller particles it is expected that turbidity will be equal or higher (lower transparency=lower Secchi disk values) after the drum filter compared to turbidity before the drum filter, because mechanical filtration will often break down the larger particles into smaller ones. In this experiment the opposite is observed; turbidity is lower after the drum filter than before. This complies with the findings of earlier experiments at Zeeland fish and will support the hypotheses that at periods when levels of settable solids and the larger suspended solids are high the larger particles will help mechanical filters to remove the small-suspended solids. This because the larger particles will absorb a part of the small suspended solids, which will then be able to be removed while they otherwise would pass through the much larger mesh size of the screen.

Dry matter content of the culture water at all locations over a 24-hour period is shown in figure y5 appendix F. Values of dry-matter before and after the drum are already discussed. Dry-matter content of the culture water after the trickling filter is going up and down, which suggest that the trickling filter does quickly change in its contribution regarding suspended solids between a net capture and production towards the culture system. This could be possible, but seems unlikely and with the limited amount of data no hard conclusion can be drawn. Looking back, it would have been better to sample through a 48-hour period trying to capture two cycles, which would have given us more information about the time-lag between feeding, faeces production and appearance of suspended solids.

3.7.4 Conclusions

No relation between feeding time and amount of particles between 3 um and 40 um could be found during the 24 hours period. A clear relation could be found between dry matter content before the drum filter and time of feeding. During feeding dry matter content was higher. With the limited amount of data no conclusion could be drawn regarding the contribution of the trickling filter. Transparency or turbidity does relate with time of feeding. During feeding turbidity was lower indicating a lower concentration of small particles, in between feeding periods transparency is low indicating a high amount of fine suspended solids. Care should be taken by interpreting and compare the data obtained with the different methods used to asses content and size distribution of suspended solids. It is concluded that in between periods of feeding would be the most optimal time to add the flocculants.

3.8 Testing flocculants using a pilot system

3.8.1 General objective

Determine efficiency of the drum filter to capture flakes formed by the addition of a primary coagulative and flocculant aid.

3.8.2 Layout of system

The layout of the pilot system build to evaluate the efficiency of the drum filter to remove the flakes is shown in **figure J1 appendix F**. It's a simply system, which consists out of a drum filter and two tanks. Important to notice is the installation of a sheet in the pump tank, which will prevent direct mixture of treated and untreated water.

As a result of the toxicity tests the combination of Sachtoklar P as a primary coagulative and anionic Synthofloc as a flocculate aid is chosen to achieve flocculation in this experiment. The primary coagulative Sachtoklar P is added in the so-called dose tank. Important is that the high level of turbulence in this tank will achieve optimal homogenization of the coagulative. Addition of the primary coagulative took place by using a peristaltic pump with four little hoses. The flocculation aid is added in the drain of the dose tank by using a simple siphon. It is important to add the flocculation aid a little later after the electrostatic coagulation induced by the primary coagulative took place. The primary process, electrostatic coagulation, needs reaction time. This is not necessary for the flocculation aid, which works instantaneously. After addition of the flocculation aid the water runs through the drum filter and back into the pump tank.

3.8.3 Specific objectives

- Evaluate the removal capacity of the drum filter at a low and high hydraulic load
- Comparing the removal capacity of the drum filter using Sachtoklar P separately and using a combination of Sachtoklar P and Synthofloc
- Determine pH and aluminum concentration before- and after the drum filter

3.8.4 Preliminary experiment

3.8.4.1 Material and methods

Preliminary experiments were executed to test if the set-up of the pilot system was suitable to test the flocculants. Important is to evaluate if flakes are formed, the amount of flakes formed and if the flakes formed will arrive at the drum filter. The difference in removal efficiency and pressure before and after the drumfilter using high and low hydraulic loadings was also evaluated. Dry matter content was estimated by using filter paper. The weight of the filter paper before filtering the sample was deducted from the weight of the filter paper after filtering the sample and drying of paper. The difference is assumed to be dry matter content.

3.8.4.2 Final experiment

The removal capacity of the drum filter using Sachtoklar P separately and in combination of with Synthofloc was compared using different hydraulic loadings. Removal capacity was evaluated by determination of dry-matter content and aluminum concentration before and after the drum filter. The experimental procedure contains the following steps:

1. Water from the culture system is transported towards the pilot system
2. Start-up of pilot system
3. Measuring pH
4. Sample point A (Control). Water samples are taken in triplicate.
5. Addition of primary coagulant (Sachtoklar P (0.5 ml/sec ≈ 6 drops/l, flow 2.5l/s)
6. Sample point B (water samples directly after flocculation).
7. Sample point C (water samples after drum filter 60 seconds after flocculation and measurement of pH.
8. Addition of the flocculation aid (Synthofloc)
9. Sample point D (water samples after the drumfilter 60 seconds after adding Synthofloc and measurement of pH.

   All samples were taken in triplicate.

3.8.5 Results

3.8.5.1 Preliminary experiments

Using the peristaltic pump to add primary coagulants worked fine. Flake forming ability of Nicasal appeared to be very good. Flakes taken before the drumfilter appeared to be smaller than the flakes originally formed in the dosing tank. Most likely degradation does take place during transport to the drumfilter due to turbulence.

The drumfilter removed the majority of the flakes, but a significant part went through the filter and was clearly noticeable in the effluent of the drum. Flakes in the effluent were pumped back in the pump and dosing tank, but degraded due turbulence in apparently such a way they were not able to form flakes again. The removal efficiency did not differ much between a high and low hydraulic loads although the impression was that using a low hydraulic load seems better.

3.8.5.2 Experiments using Sachtoklar P and Synthofloc

3.8.5.2.1 Experiment A: using low hydraulic loading

Dry-matter contents before adding the primary coagulant, before and after the drumfilter (after adding the primary coagulant with and without Synthofloc) are shown in **figure U1 (Appendix F).** Dry matter content increased from approximately 80 mg/l to approximately 250 mg/l as soon as the primary coagulant is added. This can only partly explained by the addition of the coagulant itself as only 13 mg/l (0.5 ml/2.5l=0.2ml/lx1.23=0.246 g/lx0.053 (Al salt concentration is 5.3%)=13 mg/l) of dry matter is added.

Evaluating the method used to determine dry matter content in this experiment the hypothesis that before the coagulant was added most of the dry matter content of the culture water (algae and bacteria) went through the filter used to capture dry matter, while after adding the coagulative the bacteria and algae were captured in flakes, which are all completely captured by the filter and thereby increasing dry matter values determined seems very plausible. Dry matter content after the drumfilter is higher than the dry matter content before adding the primary coagulant although the water seems more transparent, which is most likely due to the same hypotheses mentioned earlier.

As shown in **figure u1** dry matter content after the drum is 50% less than before the drumfilter when using Sachtoklar P separately. When using a combination of Sachtoklar P and Synthofloc dry matter content is 60% less. Changes in pH and Aluminum concentration are shown in **table u1.** PH level decreases with 0.7 units due to addition of Sachtoklar P and although a pH of 6.9 is within safety levels a decrease of 0.7 units in a relatively short time could cause stress for sole. The aluminum concentration after the drum is with 0.6 and 0.5 mg/l for respectively Sachtoklar P separately and a combination of Sachtoklar P and Synthofloc significantly lower than 2.8 mg/l estimated before the drumfilter. Assuming all aluminum will be in the center of the flakes it can be concluded that 79% and 82% of the flakes are removed by the drum filter for respectively the flakes induced by Sachtoklar P separately and the combination of Sachtoklar P and Synthofloc. The removal efficiency using this estimate is higher than the results using dry matter content.

The aluminum concentration measured after the drumfilter is approximately two times lower than the MTR value of 1.23 mg/l (maximum total exposure risk) for aluminum accounting for Dutch surface waters. With this concentration for aluminum no acute toxicity risk is expected for the sole in the grow-out areas.

Table u1: Changes in pH and Aluminum concentration.

	pH	**Aluminum**
Before addition of coagulative	7.6	0
Influent drumfilter after adding primary coagulative	6.9	2.8
Effluent drum after adding primary coagulative	6.9	0.6
Effluent drum after adding both primary coagulative and flocculant aid.	6.9	0.5

3.8.5.2.2 Experiment B using high hydraulic loading

Dry matter contents are shown in **figure u2 (Appendix F).** Due to extensive cleaning of the raceways dry matter content of the culture water used in this experiment was higher than the water used in the previous experiment. Due to problems with the overflow of the drumfilter during the experiment using the combination of Sachtoklar P and Synthofloc, causing flakes to go directly into the pump tank (sample point after the drumfilter) and therefore influencing the measurements after the drumfilter, the data of this experiment is not reliable, but can still be used as indication. No overflow problems occurred during the experiment when using Sachtoklar P separately.

Results regarding the removal rate at high hydraulic loadings were 48% and comparable with the 50% found in the first experiment using a low hydraulic loading. Changes in pH and Aluminum concentration are shown in **table u2.** PH level decreased with 0.7 units due to addition of Sachtoklar P, which is the same decrease as the change found in first experiment. The concentration of aluminum after the drum is with 1.9 mg/l lower than the concentration of 2.8 mg/l found before the drumfilter. Again assuming all aluminum will be in the center of the flakes it can be concluded that 70% of the flakes are removed by the drum filter when using Sachtoklar P separately. The removal efficiency of 70% using high hydraulic loadings is therefore less than the efficiency of 79% using low hydraulic loadings. The removal efficiency found using the combination of Sachtoklar P and Synthofloc is 55%, which is lower than the percentage found in the first experiment, but this is expected considering the problem with the overflow of the drumfilter.

Table u2: Changes in pH and Aluminum concentration at high hydraulic loadings

	PH	**Aluminum**
Before addition of coagulative	7.5	0
Influent drumfilter after adding primary coagulative	6.8	2.7
Effluent drum after adding primary coagulative	6.8	0.8
Effluent drum after adding both primary coagulative and flocculant aid.	6.8	1.2*

*Value not reliable due to overflow of drumfilter.

3.8.6 Discussion

The aluminum concentration shown in **table u1 and u2** are higher than the concentrations measured. During validation of the method it appeared that the method to analyze aluminum did underestimate the real concentrations. The values needed to be corrected.

As a high hydraulic loading will decrease the efficiency of the drumfilter to remove the flakes and also increase the risk for overflow of the drumfilter it became clear that it might be better to design a by-pass for removing suspended solids by using flocculating agents. A by-pass will allow lower hydraulic loadings than hydraulic loadings necessary for maintaining basic water quality parameters of the culture system itself. Lower hydraulic loadings in the by-pass will increase efficiency, but also decrease the effect of the flocculants used on pH and aluminum amount on the total culture water (and concentration). The expectation is that the total removal capacity could be further enhanced by the insertion of a plate separator in the by-pass system just before the treated water goes into the drumfilter. Normally plate separators are not very efficient in salt water systems, but the sedimentation characteristics observed for the flakes formed when using the combination Sachtoklar P and Synthofloc are good enough not to cause problems.

3.8.7 Conclusions

- The aluminum concentration gives a better estimate of the removal efficiency than the dry matter contents.
- Sachtoklar P in combination with Synthofloc does show the best results regarding the removal efficiency of both the flakes as the aluminum.
- The removal efficiency decreases at higher hydraulic loadings.
- Aluminum concentrations measured after the drumfilter are lower than the MTR value, no acute toxicity risk is expected for the sole in the grow-out areas.

3.9 Forecast of aluminum concentration in recirculation systems

Assuming a removal efficiency of 80% for the flakes for a good working drumfilter. It is easy to make an estimate of the development of the aluminum concentration of the culture water in the system when the amount added is known.

The necessary dose of the primary coagulant Sachtoklar P when used in combination with Synthofloc is estimated to be 0.164 g/l (4 dr/l). Aluminum concentration of Sachtoklar is 5.3% or 8.69 mg/l. Assuming that 80% of the induced flakes are removed by the drumfilter it can be estimated that the Aluminum concentration is 0.2x8.69=1.74 mg/l. Treating all water of the culture system the aluminum concentration will increase towards the maximum of 1.74 mg/l. Suppletion level of a system with a volume of 200m3 is approximately 2 m3/hr and will cause concentration to decrease. Assuming aluminum can only be removed due to suppletion the course of the concentration is shown in **figure u3 appendix F.**

For practical implementation the course of the aluminum concentration when treating only 25% of the culture water is most relevant. At this treatment level aluminum concentrations stay within safety limits when treating the whole volume the aluminum concentration at the beginning of the treatment will be above the MTR safety level, excluding practical application.

3.10 Methods of application and costs

Table 2 does show an overview of the estimated yearly costs for using a primary coagulative separately or in combination with a flocculate aid when treating 25% of the culture volume on weekly basis.

It is important to notice that the use of a primary coagulative separately is always more expensive than using the primary coagulative in combination with the flocculate aid. This because the use of flocculate aids will significant decrease the necessary dose of the primary coagulant, which is the most expensive agent, to induce sufficient flocculation. The single use of Nicasal appears to be the most expensive option. The combined use of a primary coagulate with Wisprofloc is more expensive than with Synthofloc, mainly because 20 times more Wisprofloc compared to Synthofloc is necessary to achieve the same results. Even tough Wisprofloc is less toxic and the total price difference between Wisprofloc and Synthofloc is limited the use of Synthofloc is preferred, because Wisprofloc will increase the organic load and therefore bacteriological growth within the system. Besides the price there is a minimal quantity of delivery. For Wisprofloc the minimal quantity is 300-360 kg, enough for approximately 7 years, which brings the products also over its use before date. Price wise the combination of Sachtoklar with Synthofloc, which will cost 350 euro/year would be the best option, but toxicity of Sachtoklar is higher than Sachtoklar P. Therefore the combination of Sachtoklar P with Synthofloc is preferred even though the costs are significantly higher (719 euro/year). To be able to quickly calculate the cost of alternative flocculate agents or treatment frequencies not tested during this project a model was set-up, which can be found in the appendix.

Table 2: Overview of expected costs by a weekly treatment of 25% of the culture water

Product	Cost per year
Sachtoklar	505
Sachtoklar P	1058
Nicasal	1169
Combination of Sachtoklar and Synthofloc	350
Combination of Sachtoklar P and Synthofloc	719
Combination of Sachtoklar P and Wisprofloc P	797
Combination of Sachtoklar P and Wisprofloc N	784

Conclusion
Sachtoklar P in combination with anionic Synthofloc is preferred to induce flocculation in culture systems.

3.11 Final conclusions and recommendations

- Flocculation can facilitate removal of (small) particlesSachtoklar P in combination with anionic Synthofloc is the best choice considering efficiency, toxicity, organic loading and costs
- When treating less than 25% of the culture water no acute toxicity risk is expected for the sole in grow out areas
- Flocculation seems to be an interesting practical method for managing turbidity, but further research is needed before technology can be implemented

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