IOSI PROJECT FINAL TECHNICAL REPORT

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EXECUTIVE SUMMARY

Oligochaete worms are naturally present in Canadian soil and were already known to (at least temporarily) survive in oil sands tailings, accelerating tailings dewatering and consolidation and improving strength properties. The latter were the main conclusions from the previous work executed by a collaborative research project between Deltares and Univeristy of Alberta, but for a range of parameters that was considered not representative of in-situ conditions (i.e. initial S_c (Solid content)and type of tailings used). In the current project, we have refined the operational parameters (we used fresh TT and FFT at their in-situ densities, and we varied temperature) and we have scaled-up (to 1.85 m columns), linking with further research efforts prior to pilot implementation. The project consisted of a series of beaker tests, small-scale columns, and large-scale columns to address the objectives of:
1) Quantifying improvements in dewatering properties and strength development produced by *Oligochaete* worms (in the absence of other additives) in fluid fine tailings FFT and thickened tailings TT, both in small-scale and large-scale tests;
2) Understanding and characterizing environmental conditions or additives that optimize *Oligochaete* worms survival and enhance reproduction in FFT and TT;
3) Quantify the effect of the environmental conditions as obtained in 2) in the dewatering and strength development properties as studied in 1), for FFT and TT, again in small-scale and large-scale tests.

Overall, worms in the absence of additives deliver relevant dewatering and strengthening improvements compared to the controls with no worms. In the small scale, these improvements are typically a factor 2 larger than improvements induced in the controls by self-weight consolidation only. This is confirmed by an increase in S_c (which also occurs faster) twice as large as in controls and by undrained shear strengths that are twice as large in their absolute value as in controls too. Direct measurements of permeability with and without worms confirmed that given a certain void ratio (which was observed during the experiments, thus belonging to the parameter space of operations) the permeability of worms treated beds can become up to 2 orders of magnitude larger than these of the exact same void ratio in the absence of worms. This enhanced permeability decreases as the void ratio decreases, but it is probably responsible for the superior dewatering and strengthening observed under worms treatment. *Tubifex* worms work best under the absence of other treatments, delivering the typical factor 2 improvements regardless of the type of tailings. *Lumbriculus Variegatus* (in this report *LV*), which is the only feasible option in Oil Sand Tailings given Canadian legislation, are characterized by increasing tailing property improvements a factor 1.3 to 1.75, depending on the tailings type. Temperature variations were also addressed, finding that decreasing it from 24 °C to 10 °C did not change the magnitude of the beneficial effect of worms. In the large-scale tests, where only *LV* worms were studied for FFT and TT, worm treatment resulted in dewatering improvements in the same order but smaller than in the small-scale tests. Nevertheless, the experiments in the large scale were interrupted after 4 months as planned, but without having reached equilibrium, suggesting that further improvements for worms only in the large scale are possible (even when worms have already deceased, which by the way was not the case in all large-scale columns with only worms, and as the enhanced permeability of the bed due to the worms tunnels remains useful for dewatering after the worms disappear).

Subsequently, we executed a beaker test study to assess the usage of potential additives to ensure worms reproduction and survival in the long term. Our results showed that after 4 months the absence of any additive resulted in a decrease of worms population around 50%, hence better but similar to the numbers obtained in previous studies. Moreover, the addition of low-quality

organic matter, particularly straw pieces of 3 cm in our tests, leads to a factor 3 increase in worms population after 4 months. Note that we foresee applications of the technology where worms reproduction and/or long term survival is not necessarily needed (e.g. mine closure and capping), but it always remains useful to be able to know and to control the amount of worms alive in the system. The survivability tests were executed for FFT only, and were independent of the type of worm used. The fact that *LV* and *Tubifex* showed similar reproduction and survival with and without additives in FFT justifies the choice of *LV* as new worms for this study after the scope modification.

When straw is added to the small-scale tests, consolidation speed and amount remains as good as it was with worms only, and in one tailings type (TT) worms managed to reproduce beyond their original numbers. The reproduction rate was not as large as predicted by the beakers tests, but this is understandable as the amount of straw in the tailings had to be decreased when transiting to the large scale, and since the tailings used in the large scale tests were 2 years older than in the beakers (different tailings could require a different recipe). Moreover, worms managed to increase the undrained shear strength in small-scale tests, also approximately a factor 2 from what exhibited by a tailings bed that contains straw (and which is in the order of several 100 Pa). The study of the large-scale tests (1.85 m large columns) revealed the superiority of the straw and worms treatment over all the studied controls, including the one with worms only. This is a difference with the small-scale results. In particular, worm and straw treated columns resulted in 5 to 10% further consolidation in absolute value than the control with no treatment or additives (e.g. 30% consolidation measured for treatments, 20% for controls, thus a 10% difference). These values were larger in the small-scale tests for worms and straw treatments (where we had 10 to 17 % further consolidation in absolute value), but as indicated before the large-scale tests were interrupted before equilibrium was reached. Moreover, and similar to what observed in the small-scale tests, the undrained shear strength was found to be larger than in any of the controls over the entire depth of the column (clearly for TT, only marginally for FFT), suggesting an impact of the worms beyond their initially attributed surficial effect. Nevertheless, methanogenesis was observed in all large-scale columns treated with straw, which ended up killing all worms which therefore most likely diminished the beneficial effect of worms. Mastering the design of the treatment recipe (in fact for every new type of tailing to be tested), in particular the % of straw or other amendments to be added, is an important aspect in order to unveil the maximum potential of worms to treat thick layers of tailings, which nevertheless seems to be significant enough for the status of development of the technology as tested in this study (e.g. with an amount of straw that, in the large-scale, delivers best dewatering results but no long term survival of worms).

Summarizing, the technology has demonstrated a relevant performance when tested in thin layers of 30 cm under operational parameters (tailings type, solid contents, and temperature) given the measured enhanced permeability by the worms, and a mechanism to ensure the survival of the worms in the long term has been identified via the addition of straw at 3% in weight of dry solids and smaller. Incorporating this mechanism into the 30 cm experiments resulted in a similar performance of the technology and in worms reproduction, but not reaching the reproduction numbers that were expected. The latter was likely caused by the adjustment of the amount of straw when applying it at a somehow larger scale. In the final 1.85 m columns, the treatment of worms plus straw exhibited the largest consolidation, the largest strengths, and the largest solid contents among all large-scale tests. Improvements by the treatment decreased from what measured in the 30 cm layers, but the experiment was stopped before equilibrium and methanogenesis occurred due to sub-optimal design of the amount of straw for a new type of

tailings. We presume that continuing the large-scale experiments until reaching equilibrium and properly designing the amount of straw as a function of the tailings type to be treated will provide an actual quantification of the potential of worms to dewater 2 m layers of tailings and larger.

Multiple worms, some not applicable in Alberta, where tested in the small-scale tests, but the large-scale results where only performed for worms that could potentially be used in applications. Nevertheless, the research team does still strongly suggest to look for local possibilities of worms, and given economic and ecological considerations.

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1 INTRODUCTION

Oligochaete worms are naturally present in Canadian soil and has been proven to (at least temporarily) survive in oil sands tailings, accelerating tailings dewatering and consolidation and improving strength properties at equal water content. The latter are the main conclusions from the previous work executed by a collaborative research project between Deltares and University of Alberta. After gathering scientific evidence on the beneficial use of *Oligochaete* worms for oil sands tailings dewatering and consolidation, a natural subsequent step is to refine the operational parameters and to scale-up prior to pilot implementation. Leveraging the on-going test program, this project proposes to continue with beaker, small-scale columns, and large-scale columns to test the objectives of:

1) Quantifying improvements in dewatering properties and strength development produced by *Oligochaete* worms (in the absence of other additives) in fluid fine tailings FFT and thickened tailings TT, both in small-scale and large-scale tests;
2) Understanding and characterizing environmental conditions or additives that optimize *Oligochaete* worms survival and enhance reproduction in FFT and TT;
3) Quantify the effect of the environmental conditions as obtained in 2) in the dewatering and strength development properties as studied in 1), for FFT and TT, and both in small-scale and large-scale tests.

While Objective 1 is the main objective of the study, which justifies application of this technology, Objective 2 and Objective 3 produce critical information about feasibility and potential to effectively scale up to operational conditions. This technology offers a natural method to improve oil sands tailings management which can be employed on its own, or coupled with other technologies, such as sand capping or vegetation planting.

The scope of this study is divided in four tasks (Task *i*) with specific targets, all of which contribute to these main research objectives.

- Task 1: small-scale column testing of selected parameters to focus existing findings to realistic environmental conditions, in particular tailings type (FFT and TT) and temperature;
- Task 2: series of simple beaker tests including biochemical parameters (i.e. nutrients and organic matter availability) to explore optimization of *Oligochaete* worms reproduction in oil sands tailings, to ensure sustainability of the technology;
- Task 3: second series of small-scale column tests under the effect of controlled additions of nutrients and organic matter (case where beaker tests were found to stimulate Oligochaete worms reproduction); and
- Task 4: large-scale column testing using optimal parameters from Tasks 1-3 to provide conditions for follow-up pilot implementation.

Note that during the previous phases of this research program, *Tubifex* was the *Oligochaete* worm selected for treating tailings. This was motivated by *Tubifex* remarkable resistance to toxicity and anoxia. The current IOSI project and all its tasks were also envisioned for *Tubifex*, which was tested over the first batch of laboratory experiments. However, in May 2018 Canadian legislation prohibited the commercial distribution of *Tubifex*, as it is carrier of the whirling disease which is dangerous for salmon. At this point, the research team and the project stewards

discussed and agreed to continue the research program with *Lumbriculus Variegatus* (a.k.a. California Blackworm; *LV* in this report), who can also survive in harsh conditions and contaminated sludge (Hendrick et al, 2007; Ellissen, 2007), and endemic to Alberta as well (Clifford, 1991). Partly funded by IOSI (repetition of Task 2 for *LV* worms) see IOSI2016-08-Tubifex_ScopeChangeProposal_20180730) and partly by Deltares own contribution (quantification of *LV* performance on solids content (S_c) improvement in Task 1; inclusion of *LV* in Task 3), the fundamental experiments in the current IOSI project were repeated for *LV* as well. Over the next paragraphs all tasks are presented, including their objectives, an introduction of the experiments performed to address these, and the deliverables per task.

1.1 Task 1: small-scale column testing for environmental parameters

Objective

The objective of this task is to quantify the dewatering and strengthening capacity of worms when applied for a selection of operational parameters (characteristic tailings density and type, temperature, etc). This task included a number of small column tests (10 cm diameter, 1 m tall) at the Deltares Sediment Laboratory. Tests lasted up to four months. Each parameter was tested in duplicate to account for natural variation, which is expected in presence of living organisms. These parameters were chosen based on the results of the current research program described in the background section. The parameters that were tested are:

- Different tailings types and initial solids content chosen in agreement with industry, i.e. FFT and TT, with and without worms at the reference worms density.
- Different temperature in one tailings type at the reference worms density.
- An initial concentration of each of the studied tailings types following the in-situ characteristics of tailings.

Experimental set-up

The experimental set-up is discussed in depth in section 3. Here a summary of the executed tests to address the Task's objective is given:

- Tailings characterization (e.g. PSD, Water Content, Density, Atterberg) before and after the test when suitable and feasible;
- Automatic high-resolution pictures of all columns at established interval, to track mudline evolution;
- Vane testing for strength before and after the test (profile with depth);
- Falling head testing on a set of separated columns. It was first envisioned to install the falling head necessary hardware at a selection of the settling columns to be studied in this task, enabling to perform direct measurements of permeability during the dewatering and consolidation standard tests. However, the authors hypothesize that the downwards flow of water behind the measuring principle of the falling head could interfere in the rate of dewatering (upwards water flow) of the tailings. Therefore, a separated experiment was set for the falling head experiment. The installation of an independent falling head set up was fully funded by Deltares.

Deliverables

Task 1 produced a concise report that includes description of the experiment setup and results, for all columns, of:

- Sediment characterization including gelling concentration;

- Solids content (S_c) evolution in time;
- Strength and density profiles;
- Permeability as measured in the falling head tests.

The results will be presented to compare worms and absence of worms performance. The core of the results was produced for *Tubifex* prior to the modification of scope, with a final experiment to check the performance of *LV* in the S_c evolution of tailings (funded by Deltares).

1.2 Task 2: beaker testing for worms survival and reproduction

Objective
The objective of this task was to explore the possibility to optimize the survival and reproduction of oligochaete worms in oil sands tailings. The latter is done by adding essential nutrients and/or food for the worms. Both the essential nutrients and the food will be regarded as additives in this report. In a final experiment at this task, the survival and reproduction of worms in unsaturated conditions was studied for the first time.

Experiments prior to this project showed worms survival in tailings, but only minor reproduction. In addition to being a rather toxic environment, tailings have proven to be nutrient scarce. Bacterial degradation of residual hydrocarbons may be stimulated by offering inorganic nutrients in the tailings. This could be achieved rather cheaply using agriculture fertilizers. Alternatively, the addition of organic matter can also stimulate the reproduction of worms. We explored the addition of high-quality organic matter (freshwater micro-algae) and the addition of low-quality organic matter (straw or hay, with or without addition of inorganic nutrients). They used one type of tailings (FFT or TT) only. Originally Task 2 was meant to have *Tubifex* only as subject of the study. However as mentioned over the Introduction section *Tubifex* had to be exchanged by *LV* because of developments in Canadian legislation. To give completeness to the study initiated to *Tubifex* in the previous in-kind phases of this study, experiments in Task 2 were repeated for *Tubifex* as well. Task 2 is then completed with a final task where additives are not introduced, and where we studied the effect of unsaturated conditions in worms reproduction. Overall, the sub-tasks in Task 2 read as follows:

- Task 2A: beaker tests with additives for *Tubifex* survival and reproduction.
- Task 2B: beaker tests with additives for *LV* survival and reproduction.
- Task 2C: explorative beaker tests for worm survival in the absence of additives and under non-saturated conditions.

Experimental set-up
Tasks 2A and 2B consist of beaker tests (containing 500 ml of sediment per beaker). The additives that will be tested are:

- Inorganic nutrients.
- High quality organic matter.
- Low quality organic matter.
- Inorganic nutrients in combination with high quality organic matter.

At predetermined time intervals (3, 6, 16 weeks), one series of beakers were sacrificed. Monitoring will only be done upon scarification of a test and consists of sieving the content of the beakers to retrieve and count the number of live oligochaete worms.

Task 2C on the other hand was just an explorative study to assess the effect of an non-saturated interface in the reproduction of worms. Identical tailing volumes and worm concentrations were studied, but with only one counting moment after 3 weeks, and with no replicates.

Deliverables
Task 2 produced a report with the experimental setup and results of the beakers tests. Specifically, this report will attempt to produce a conceptual understanding of environmental conditions that influence and optimize worms survival in oil sands tailings.

1.3 Task 3: small-scale column testing with for environmental parameters with additives

Objective
The objective of Task 3 was to test the effect of the findings from Task 2 in a selection of the parameters tested in Task 1. In practice this means to study the performance of the worms survival strategies in a realistic parameter space. As it also occurred in some of the experiments in Task 1 and in the experiments of Task 2, these experiments were executed twice, for *Tubifex* and for *LV*. Deltares funded the repetition of the tests, as otherwise the *Tubifex* study would have been incomplete, without one particular species covering the complete experimental program. We believe that not having a fully completed experimental program for each of the species would make positioning – understanding of the results more difficult, likely contributing to uncertainty when evaluating the potential of the only valid implementation alternative: *LV*.

Experimental set-up
This task used the same type of columns available for Task 1 with the aim of understanding the consequences of the application of successful (in triggering worms long term establishment) additives. All experiments lasted for about 16 weeks and were sacrificed (e.g. worms were counted) at the end. Only a minor (S_c and strength) but still relevant set of measurements were done to the columns in Task 3.

Deliverables
Deliverables from Task 3 updated the parameters reported in Task 1 for the effect of additives (with and without worms), focusing at the sustainability of the technology at operational scale. The task will also indicate which parameters to further test in large-scale column tests (Task 4).

1.4 Task 4: large-scale column testing
Prior to the execution of the large-scale tests, a number of preliminary tests were executed to test parameters reported from Deltares as a result of their work in Task 1-3. The latter was motivated by a) potential differences between the worms in North America and Europe, and b) possibly different type of tailings. Experiments are a simpler version of the experiments performed by Deltares in Task 2.

Objective
This task had the objective of bridging the small-scale column experimental plan with pilot implementation, as it included executing and analyzing four large-scale column tests (0.12 m diameter, 1.85 m tall). The input parameters for the experiments will be based on the findings of Tasks 1-3, which will determine the exact parameters and conditions to be tested. Experimental

monitoring in the large-scale columns linked geotechnical considerations of strength and solids content with environmental conditions for *LV* survival and any improvements in released pore water chemistry as a result of biodegradative processes. These tests are carried out in the University of Alberta Applied Geo-environmental Research Facility.

Experimental Setup

These tests utilized ten large-scale columns. The duration of each test was of at least four months (eight months total for four tests). The columns are:

- Ten Townsend acrylic columns at 2.5 m tall, 0.305 m diameter and 0.635 cm wall thickness; and
- Ten sampling ports, two each at heights of 0.40, 0.80, 1.20, 1.60 and 2.00 m, controlled by Swagelok SS Quarter-Turn Instrument Plugs, model SS-4P6T4.

Experimental monitoring included:

- Interface level of water and tailings deposit;
- Geotechnical characterization (solids content, hydraulic conductivity, undrained shear strength (peak and residual), Atterberg limits, particle size distribution);
- Pore water pressure
- *LV* worms survival;
- Microbial community characterization through 16S rDNA pyrosequencing; Institute for Oil Sands Innovation June 2016
- Overlying water and pore water characterization (redox, pH, alkalinity, chemical oxidation demand, biochemical oxygen demand, naphthenic acids, dissolved organic compounds, MicroToxR)

The tailings thickness in these columns will be of 1.85 m. Thisallowed for observation of *LV* penetration depth beyond the current maximum of 42 cm.

Deliverables

Task 4 produced a concise report with the experimental setup and results of the large-scale column tests. It is expected that this task will produce results on achievable solids content and strength as well as information about (optimizing) *LV* survival.

2 BACKGROUND

This section reads over the findings of the previous phases of the project, and thus over results obtained for Tubifex as indicated in the introduction section. Though not part of the plans for scaling up this technology anymore, *Tubifex* constituted the first species ever tested in the context of this technology, and it will be therefore still introduced in depth as it reflects the qualities and characteristic that a species of worm used for dewatering of tailings should have. *Tubifex* (*Tubifex tubifex*, or *T. tubifex*) are, as the current focus of this project *Lumbriculus* Variegatus, an *Oligochaete* worm. *Tubifex* belongs to the *Tubifex* genus, which, also as the current focus *Lumbriculus Variegatus*, is indigenous in Alberta, Canada (Brinkhurst, 1978; Whiting and Clifford, 1983). *Tubifex*, also known as sludge or sewage worms, usually inhabit the bottom sediments of lakes, rivers, and occasionally sewer pipelines and outlets. The worms ingest sediments, selectively digest bacteria and degradable organic matter, and absorb molecules through their body walls. They feed below the sediment surface and defecate on the sediment surface behaving as bioturbation agents (Davis, 1974; Fisher et al., 1980). The worms can survive in limited oxygen environments by waving hemoglobin-rich tail ends to exploit available oxygen. It was found that *Tubifex* are abundant in organic rich waters because of lack of competition and abundant food supply, in conjunction with a high tolerance for reduced oxygen conditions (Chapman, 2001). They can survive in severely polluted environments where almost no other species can endure (Engle et al., 1994). Due to their high resistance to pollution, *Tubifex* density has been used as a bioindicator of pollution caused by heavy metals such as copper and lead (Lucan-Bouché et al., 1999). In recent years, *Tubifex* have been successfully used as a biological remediation method for removing environmental contamination in biological wastewater treatment processes (Huang et al., 2007, Liang et al., 2006). In addition, rapid enhancement of the sludge settling rate was observed especially for sludge with high total suspended solids concentration. This was likely due to the tunnels made by *Tubifex*, which improved solid-water separation (Zhu et al., 2008). Research on *Tubifex* treatment of estuarine and lacustrine sediments (Rhoads and Young, 1970; Willows et al., 1998; Widdows et al., 1998, 2000; Amaro et al., 2007; de Lucas et al., 2014) has also been
done, where the sediments have similarly low solids content as the oil sands tailings. In one of these studies, *Tubifex* were shown to speed up the consolidation process of lake bed sediment because of increased permeability of the bed (de Lucas et al., 2014). Due to the positive effects on enhancing sludge settling rate and soft sediments dewatering highlighted in previous research, *Tubifex* treatment was first introduced as a potential viable, environmentally friendly alternative to the enhanced dewatering of oil sands tailings in Canada. Later in the project, and as elaborated in the introduction section, the type of worm used for dewatering and strengthening of oil sand tailings shifted from Tubifex to *Lumbriculus Variegatus*, and given both its similarities with *Tubifex* with respect to resilience and resistance (Hendrick et al, 2007; Ellissen, 2007) and its local nature (Clifford, 1991).

During the 2014 – 2016 research program, we conducted a series of small-scale laboratory column dewatering tests and survival tests on oil sands tailings using *Oligochaete* worms in combination with polymer treatments. In all tests, the *Oligochaete* worms concentration used in the column tests was comparable to field concentrations at ecologically deteriorated lacustrine systems (de Lucas Pardo et. al 2014). The most important results to date are:

- Almost all *Oligochaete* worm individuals can survive one month in both FFT or MFT at room temperature (e.g. 24 °C). Survival rate in MFT at near zero temperatures (e.g. 4 °C)

is slightly smaller, but still higher than 80%. When testing time increases to 90 days, survival rates at room temperature for FFT drop to 20 to 40% (Yang, 2016).

- A 30 cm thick 30% Sc MFT layer of *Oligochaete* worms treated tailings showed the following relative improvements with respect to non-worms (yet polymer) treated tailings (Yang, 2020): 40% relative larger increase in solids content: 50% Sc versus 43% *Sc* after two months; Note that this numbers has been largely improved under the current research program.
- A up to 60% relative larger undrained shear strength is found at depth in Oligochaete worms treated tailings. The latter is possibly due to oxidation of the sediment around each *Oligochaete* worms tunnel. This leads to a network of oxidized channels, which also improves the strength of the overall tailings layer influenced by *Oligochaete* worms.
- It was also reported that Oligochaete worms dig tunnels over the whole depth at a 42 cm thick layer, which is the thickest tailings layer tested to date.

A summary of the 2015 program, laboratory setup and results can be found in Yang et al., 2016 and Yang et al., 2020. This work builds directly upon these past experiences and findings, and intends to bring this technology a step forward towards pilot implementation.

3 EXPERIMENTAL

3.1 Materials

The following information was made available with respect to the origin of the tailings used by Deltares in Tasks 1-3:

- Both FFT and TT were provided by Exxon Mobile.
- TT was treated with approximately 75 g/t of a medium weight anionic polyacrylamide.
- The production date of the TT drum is, Jan 15/17 (22:30).
- It was treated with polymer 1 to 0 days before production.
- The type of polymer used on this drum of TT was SNF A3332.
- FFT was labelled as S1117.

As for the tailings used in the University of Alberta tests, we know:

- That we communicated the above stated information to the project stewards for them to best select a set of new tailings as similar as possible.
- That the tailings were produced almost 3 years after the batch for Deltares.
- Further details on the origin and characteristics of these other new tailings were not provided.

3.2 Task 1: small-scale column testing for environmental parameters

3.2.1 Tailings characterization

Tailings characterization consisted in the determination of the following parameters:

A) Initial solids content S_c, with: $S_c = m_s / (m_s + m_w)$, where:

- S_c: solids content (-)
- m_s: mass of solids (kg)
- m_w: mass of water (kg)

m_s and m_w were determined as follows: tailings samples were placed in containers of known weight, weighted in a scale, and placed in the oven at $105°C$ for the next 24 h. Hereafter the container was weighted a gain, with which the mass of water m_w in the sample was determined as the net difference between weights. The mass of solids m_s is the final weight minus the (already known) weight of the container.

B) Initial tailings bulk density ρ_{bulk}, with: $\rho_{bulk} = (m_s + m_w)/(\rho_s * m_s + \rho_w * m_w)$, where:

- ρ_{bulk}: bulk density (kg/m3)
- ρ_s: density of solids = 2650 kg/m3
- ρ_w: density of water = 1050 kg/m3

The mass of solids and the mass of water were determined as indicated in 3.2.1 A).

C) D_{50} of the tailing's grain size distribution.

The grain size distribution was obtained by dispersing small concentrations of tailings in water with the same salinity as tailings process water, and measuring at a Malvern Mastersizer 2000. The measuring principle of Malvern Mastersizer is laser diffraction. The small concentrations tested in the Malvern were set to the optimal operational range of the instrument. Once the

granulometry is obtained, D_{50} is defined as the size for which 50% (in volume) of the particles in the suspension are smaller.

D) Sand to fines ratio *SFR*, based in grain size distribution.
The sand to fines ratio SFR is the mass ratio of sand to fines, therefore the mass of mineral solids with a particle size larger than 63 µm divided by the mass of mineral solids with a particle size smaller or equal than 63 µm. This ratio is equal to the ratio between the sand fraction to the fines fraction, as determined from granulometry measurements, assuming that fines and sand have an equivalent density of solids ρ_s.

E) Gelling concentration c_{gel}.
The gelling concentration c_{gel} is the concentration at which the bed begins to exist. c_{gel} can be estimated by observing the settling interface of a known concentration sediment mixture. Figure 3.1 shows the transition from settling to dewatering-consolidation at a generic sediment mixture. The gelling point can be identified as the point where the slope of the settling interface changes. In Figure 3.1, this point is characterized by its height H_{gel} . For a initial concentration c_0 and a initial suspension height H_0, the gelling concentration c_{gel} can be estimated following conservation of mass as follows: $c_{gel} = (c_0 * H_0) / H_{gel}$

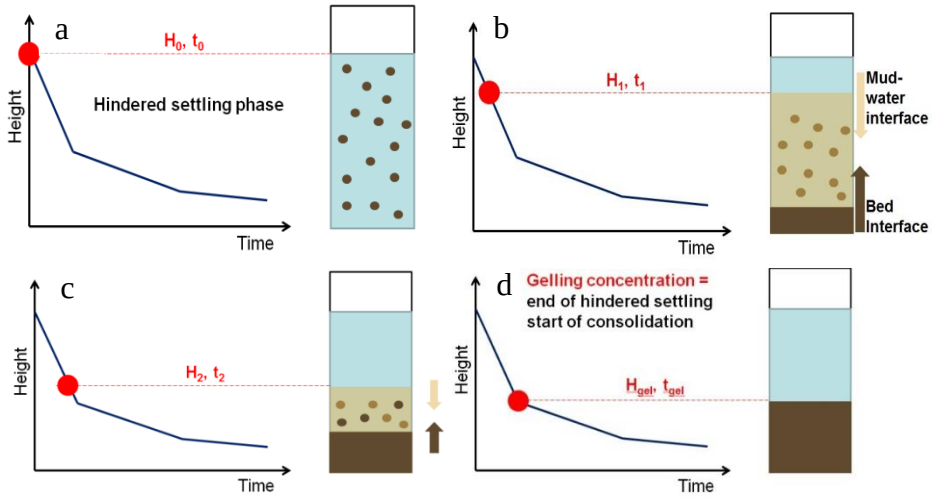

Figure 3.1. Transition from settling to consolidation.

3.2.2 Solids content evolution

In settling and consolidation experiments the mass of solids m_s remains constant over the experiment, and equals to the initial mass of solids added to the columns. This means that by keeping track of the volume of sediment over the experiment, the S_c of the bed can be computed. The volume of sediment is calculated from pictures taken by a high resolution camera. The camera automatically shots several pictures every day, with a decreasing number of pictures per day as time passes.

3.2.3 Tailings properties evolution

A) Strength:

The peak shear strength and the remoulded shear strength of cohesive sediment can be determined with a vane test. A vane (Figure 3.2) is placed into the soil and rotated with a constant angular velocity. The resistance of the sample is measured by a torque transducer between the rotating vane and motor. The recorded signal (Figure 3.2) is multiplied by the A-factor, which is dependent on the properties of the vane. The peak strength is a function of the rotation speed, stress history and the sample preparation. The remoulded shear strength is a material property at given water content of the sample and is defined as the residual stress in the bed after failure of a sample. It gives an indication of the resistance of the sediment bed to erosion. All measurements lasted for 450 s.

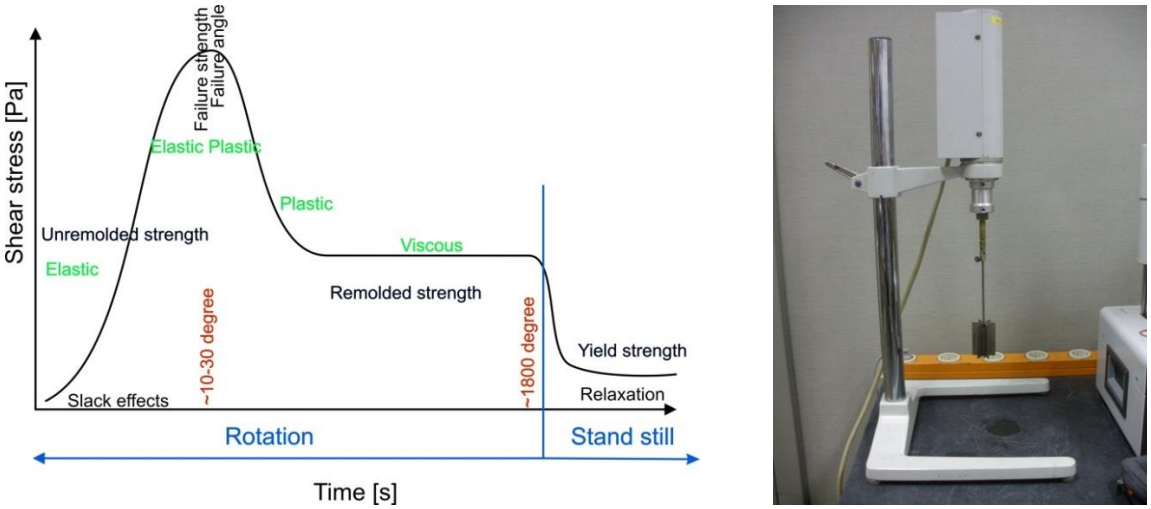

Figure 3.2 Left panel: example of vane measurement. Right panel: Vane instrument with vane element FL10.

Sediment strength (peak and remoulded) is measured with a Haake vane instrument. The Haake vane at the sediment laboratory of Deltares can be used to measure at several depths in sediment cores without sub-sampling. A more accurate and state of the art Haake Mars rheometer, also with the possibility to install a vane and measure strength, is also available at our laboratory, but requires subsampling from the bed to study strength, which is not ideal due to the fluidity of the studied tailings. However, the Haake Mars is used in advanced to verify that the Haake vane instrument does deliver accurate results for the measured tailings. Table 3.1 shows the validation of the Haake RV 100 measurements via comparison with Haake Mars measurements. Deviations between the two devices do not exceed 2.5 Pa. When averaged, results differ in less than 1.5 Pa. These deviations represent no more than 10% of the measured values, even for these very small strengths. Deviations will represent a smaller fraction of strength values for more consolidated tailings. These set of validation measurements were done in TT samples, and the results are well in agreement with what later reported as initial strength for TT as received.

	Haake RV 100	Haake Mars
	Peak strength (Pa)	
Sample1	11.545	14.01
Sample2	10.90	11.14
Average	11.22	12.58

	Remoulded strength (Pa)	
Sample1	4.77	7.28
Sample2	5.97	4.78
Average	5.37	6.03

Table 3.1. Validation of Haake RV 100 measurements via Haake Mars measurements.

B) Bulk density

Bulk density ρ_{bulk} was measured by collecting samples at several depths from the consolidation columns, once consolidation is finished, and subsequently to the vane tests. Subsampling was done at a vertical in the core that was not affected by the vane. This is because the intrusion of the bed and its rotation can modify the water content of a region of the bed, and thus its bulk density as well. Later bulk densities were obtained as described in Section 3.2.1 B).

C) Plasticity

Plasticity is study via the determination of the Atterberg limits. This experiment is outsourced to Wiertsma S.A., a geotechnical company specialized in standardized testing. Results are then plotted in the Casagrande plasticity chart, which relates plasticity index (PI) with the liquid limit (LL). Note that PI is defined as the difference between LL and the plastic limit (PL). LL and PL are direct input of the classical Atterberg limits experiments.

3.2.4 Falling head tests: permeability

The falling head test is a widely used standard geotechnical test, whose purpose is to obtain direct measurements of the permeability of a soil. Figure 3.3 shows a sketch of a falling head test, including indications to the dimensions used in this work. The heights indicated for water and soil at Column 1 are the initial heights. Water flowing through the bed in Column 1 is discharged via Column 2 into the evacuation bucket. The role of Column 2 is to provide a constant water level as reference for the actual drop in water head at Column 1. The amount of water flowing through the bed is equal to the water head difference between columns, which is used to calculate permeability as follows:

$$K = \frac{L}{\Delta t}\, ln\left(\frac{\Delta h_t}{\Delta h_{t+1}}\right)$$

K = permeability of the soil at time equals to t (m/s)
L = bed thickness at time equals to t (m)
Δt = time difference between measurements (s)
Δh_t= water head difference between top water level in Column 1 to the constant water level in Column 2 at time equals to t (m)
Δh_{t+1}= water head difference between top water level in Column 1 to the constant water level in Column 2 at time equals to $t + 1$ (m)

Note that the falling head test is meant to study the permeability of a bed in equilibrium. In this work, beds are continuously undergoing compaction. In fact, the evolution of permeability as the bed compacts (and how worms affect it) are relevant pieces of information in order to enable the transition of the technology into pilot scale. Therefore, the results of the falling head tests are not on value of the permeability K, but a series of permeabilities K as a function of the compaction state (e.g. void ratio). The diameter of Column 1 was 14.5 cm, whereas the resolution for measuring the head in the vertical at this very same column was just 1 mm. This resulted in

inaccuracies in the determination of *K* (i.e. 1 mm up or down in the water head drop has a significant effect in the calculated K*)*, but orders of magnitudes, trends, and relative differences between treatments are still properly addressed within the current methodology.

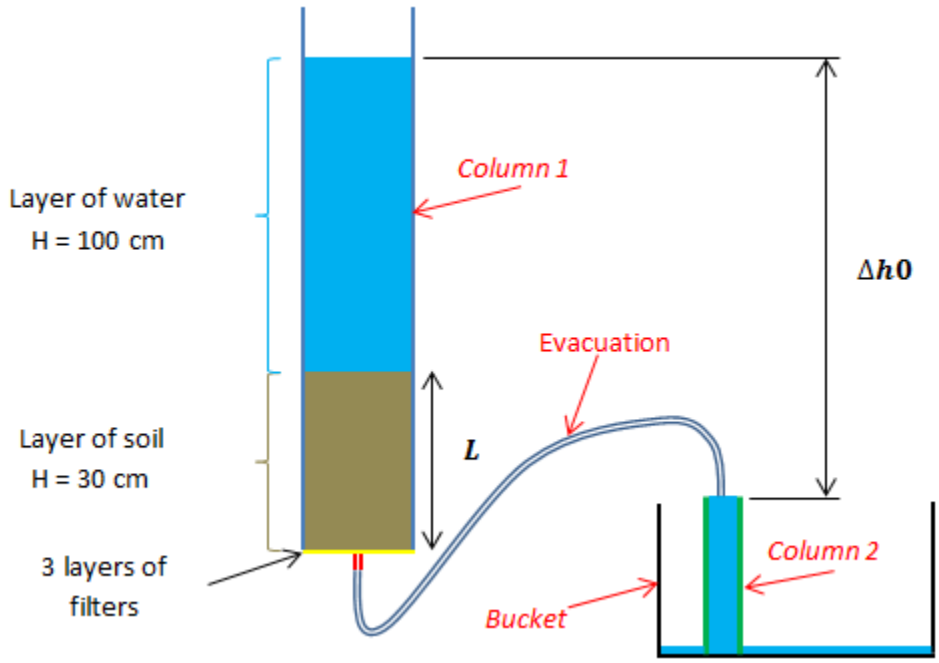

Figure 3.3. Sketch of a falling head test, with indications to the dimensions used in this work.

Permeabilities as obtained from the falling head tests are reported against the void ratios for which they were registered (i.e. the level of compaction of the bed at the moment a certain K was measured). The void ratio of a bed is defined as follows:

$e = \frac{V_w}{V_s}$, with:

e = void ratio

V_w = volume of water in the bed

V_s= volume of solids in the bed

3.3 Task 2: beaker testing for worms survival and reproduction

The methodology of each of the sub-tasks in Task 2 is not identical, as the knowledge gained in Task 2A helped designing a modified test matrix for Task 2B, with the purpose of optimizing the amount of relevant information obtained from the tests. The detailed methodology followed in both cases is introduced over the next paragraphs. In addition to the original Task 2 experiment and its repetition for *Lumbriculus Variegatus* following the scope change, we also performed an extra survival and reproduction test with the objective of assessing the effect of a non-saturated sediment interface in worms reproduction.

3.3.1 Task 2A

As mentioned in the Introduction section, in particular in section 1.2, the following additives were tested for its effect in worms reproduction and survival: low quality organic matter, high quality organic matter, and inorganic nutrients. Additives were added to 1000 ml beakers

containing 500 ml of FFT (and the remaining beaker volume was filled with process water), and 39 worms individuals. After giving time for the additive to have an effect in worms reproduction, experiments were interrupted and beakers were sieved (250 µm sieve size) and its alive worms counted under a magnifying glass. This was done 3 weeks, 6 weeks, and finally 16 weeks after the experiments started. For every additive, two identical beakers were set, in an attempt to quantify the variability of the additive performance. In particular, the additives tested (in duplicates) in Task 2A were:

- Inorganic nutrients (in fact a commercial product meant for plants which contains N, K and P) at a concentration of 1mM/beaker (IN c1 later in this report).
- Inorganic nutrients at a concentration of 0.1mM/beaker (IN c2).
- High quality organic matter, in the form of algal paste at a concentration of 3% of the dry weight of sediment (HQ om).
- Low quality organic matter, in the form of straw cut into 3 cm pieces at a concentration of 3% of the dry weight of sediment (LQ om).
- A combination of low-quality organic matter in the form of straw at 3% dry weight with inorganic nutrients at a concentration of 1mM/beaker (LQ om + IN c1).
- An extra set of beakers with no additive was also tested in parallel, and used as a reference for the performance of the additives.

3.3.2 Task 2B

In essence, Task 2B experiments were identical to experiments in Task 2A: letting additives act on worms reproduction and survival by adding them to a 1000 ml beaker with 500 ml of FFT and 39 worms. However, the number of additives tested in Task 2B was less, and experiments were only interrupted once after 16 weeks of being started. Under the assumption of *Tubifex* and *LV* reacting to additives in a similar way, we decreased the amount of test conditions following the results from Task 2A. This was compensated by setting three identical beakers for every additive. In particular, the additives tested (in triplicates) in Task 2B were:

- Inorganic nutrients at a concentration of 0.1mM/beaker (IN c2).
- High quality organic matter, in the form of algal paste at a concentration of 3% of the dry weight of sediment (HQ om).
- Low quality organic matter, in the form of straw cut into 3 cm pieces at a concentration of 3% of the dry weight of sediment (LQ om).
- A combination of low-quality organic matter in the form of straw at 3% dry weight with inorganic nutrients at a concentration of 1mM/beaker (LQ om + IN c1).
- An extra set of beakers with no additive was also tested in parallel, and used as a reference for the performance of the additives.

3.3.3 Task 2 C: non-saturated conditions

Task 2C experiments were performed in the same type of 1000 ml beakers filled with 500 ml of FFT. The initial number of worms was also 39, consistently with all other tests in Task 2. Nevertheless, for this experiments the tailings from test FFT 1 (see Table 4.1 for your reference) were re-used, instead of dedicating previously non-tested tailings to it. Experiments consisted of four beakers, two saturated and two unsaturated (by simply removing water from the top and letting the sediment interface to be exposed to air). Both *Tubifex* and *LV* were tested in both conditions, summing up to the total 4 beakers.

3.4 Task 3: small-scale column testing for environmental parameters with additives

Sediment characterization was not repeated for Task 3, as the tailings used in Task 3 are the same as studied in Task 1. Also, with respect to the evolution of tailing properties, only S_c and strength were monitored.

3.5 Task 4: large scale column testing

This Task was executed at the University of Alberta, and with the remote collaboration and supervision by the Deltares team. Prior to the execution of this task, a preliminary task with the objective of getting familiar with handling of the worms and of checking the Deltares advice for percentage of straw and reproduction was also executed for the reasons already stated in this report: the different tailings and potential differences between the same species of worms across continents. Only *LV* worms, the only viable option for implementation in Canada, were studied in the large-scale tests.

3.5.1 Column set up

For the actual large-scale column test, we constructed ten acrylic columns (12 cm ID, 1.85 m tall) equipped with 10 ports for sampling and monitoring (Figure 3.4). Ports were made out of stainless steel valves (VBSM2-075, DIRECTMATERIAL.COM) for pH and redox potential monitoring. Three columns were additionally equipped with 3 ports each for pore pressure monitoring (stainless steel valves VBSM2-025, DIRECTMATERIAL.COM). The columns were established on Nov.9, 2020 (TT) and Nov.16, 2020 (FFT). All treatments were chosen according to Deltares's recommendations, and validated and adapted during small-scale beaker tests. All columns were incubated at a temperature of +20^{o}C, straw concentration 0.2%. Worm concentration was 17 worms/L (39 worms in 2.3L in Deltares's experiment), so 374 worms in 22 L of tailings in each column. *LV* worm culture was initially purchased from Aquatic Research Organisms Inc. (USA) in December 2019 and maintained in two 10 L aquariums. The worms appeared to be healthy and active prior to release into tailings.

Cap water with modified salinity to match that of oil sands process water (OSPW) was prepared by diluting $CaCl_2$ and NaCl to mimic an ionic strength of 0.05 mol/L. This value was reported in previous studies (Pourrezaei et al. 2014; Siddique et al. 2014a, Lou et al. 2016)

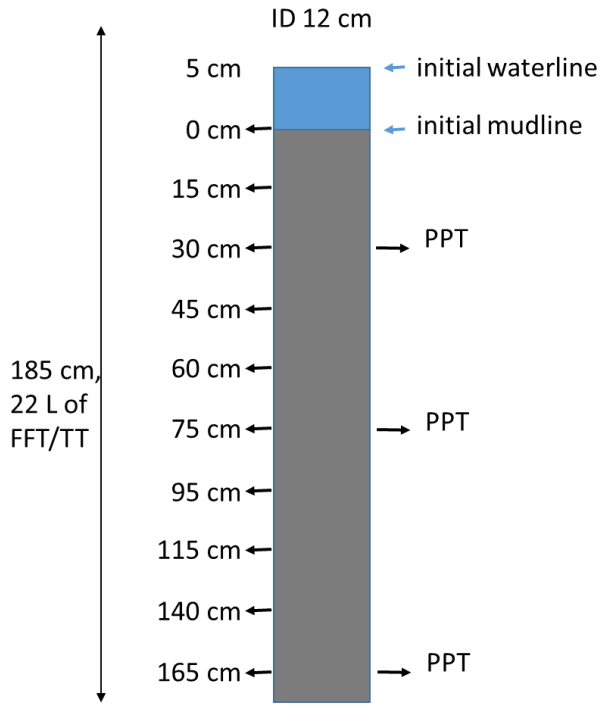

Figure 3.4. Experimental column design. Arrows on the left indicate port locations on the column; ten sampling ports for collecting tailings or cap water were installed in each column. PPT labels indicate the location of the connection of the pore pressure transducers.

3.5.2 Experimental analyses and monitoring

Following analyses were performed during incubation and at the end of the experiment:

- Interface level of water and tailings deposit;
- Overlying water and pore water characterization (redox, pH, alkalinity, chemical oxidation demand, naphthenic acids, dissolved organic compounds, Microtox acute toxicity)
- Geotechnical characterization (solids content, peak undrained shear strength, particle size distribution);
- Pore water pressure;
- *LV* worms survival;
- Microbial community characterization through 16s rRNA Illumina sequencing;

A) *Interface level of water and tailings deposit (mudline)* was measured using a ruler attached to the column. These data were used to calculate tailings consolidation. Upper water level was measured for the few first days, but then it became obvious that it was more affected by evaporation from the surface (columns on top were loosely covered by foil). Consolidation of tailings was calculated as:

Consolidation = ((Initial mudline - Actual mudline) / Initial mudline) * 100%

Interface level of water and tailings deposit was monitored every two-three days at the beginning and bi-weekly once it slowed down (after 2 weeks).

B) *The total solids content* was determined gravimetrically by drying sample at 105°C ± 2°C for the initial samples and for the samples collected at the end of the experiment for the top, middle and bottom layers of the tailings columns.

C) *Peak undrained shear strength* was determined using Brookfield Rheometer DV3T extra (Brookfield Engineering Laboratories) for the initial samples and for the samples collected from the top, middle and bottom layers during columns decommissioning at the end of the experiment.

D) *Particle size distribution* was determined gravimetrically for the initial bulk samples of TT and FFT. ASTM International D79828-17: Standard Test Method for Particle-size Distribution (Gradation) of Fine-grained Soils Using the Sedimentation (Hydrometer) Analysis using a 152H hydrometer (Fisherbrand).

E) *Pore water pressure* was monitored using Cole-Parmer High-Accuracy Pressure Transducers RK-68075-42 (Cole-Parmer) connected to DAQ973A Data Acquisition System and DAQM901A 20 Channel Multiplexer (Keysight Technologies). Pore pressure transducers (PPTs) were installed to the three columns: labeled on Figure 4.21as: (1) experiment, FFT+0.2% straw + worms; (2) control, FFT+0.2% straw; and (3) control, FFT. Pore water pressure was calculated using formula obtained from Cole-Parmer and specific for RK-68075-42 pore pressure transducers:

Pressure (kPa) = ((2*Volts) – 1)*6.89476;

F) *LV worm survival rate* was determined by manual counting of remaining worms. Tailings (the whole volume of the column) were sieved through a 150 μm sieve and worms were then counted.

G) *The microbial community structure* was studied by sequencing 16S rRNA genes using Illumina sequencing. Total genomic DNA was extracted from triplicate 0.5 g subsamples using the MP FastDNA SPIN kit for soil (MP Biomedical, OH, USA) according to the manufacturer's protocol, and then amplified using universal primers for Bacteria and Archaea with embedded Illumina indexes (An et al., 2013. Purified PCR products were sent to The Applied Genomics Core at University of Alberta (Edmonton, Canada) where the second round of amplification was conducted using Illumina bridge PCR-compatible primers followed by sequencing using an Illumina MiSeq platform (Illumina, San Diego, CA, USA). Quality-verified sequences were compared against the SILVA taxonomic database (version 3.0) and clustered into Operational Taxonomic Units (OTUs) at ≤5% distance (Dong et al., 2017).

H) *ORP and pH* were measured in the cap water and tailings through the ports using Orion Star multimeter (ThermoFisher Scientific) equipped with ORP (ORP-1, Gain Express) and pH (E-1325M, Gain Express) probes, respectively. Monitoring was conducted every two weeks.

I) *Alkalinity* was measured using a double endpoint titration with digital Eco Titrator equipped with LL-Unitrode easy Clean probe (Metrohm) for the initial samples and for the samples collected for the top, middle and bottom layers during columns decommission at the end of the experiment.

J) *Chemical oxidation demand (COD*) was analyzed using HACH COD Digestion Vials (HR 20 - 1,500 mg/L) in conjunction with a HACH Digital Reactor Box DRB200 and HACH DR 900 Multiparameter Portable Colorimeter (HACH) for the initial samples and at the end of the experiment.

K) *Naphthenic acids (NAs)* were determined using Fourier-transform Infrared Spectrometry (FTIR) (Spectrum 100 FTIR Spectrometer, PerkinElmer).

L) *Dissolved organic compounds (DOC)* were measured using a Shimadzu TOC-LCPH analyzer (Shimadzu) (sparging time: 6 min; injection volume: 50 µL; injection number: 3 out of 4; acid added: 2.3%).

M) *Microtox acute toxicity* was determined using Microtox Model 500 (Modern Water). The analysis used bioluminescence of the marine luminescent bacteria *Allivibrio fischeri* that emit light under the optimal environmental conditions. Presence of toxic substance causes a change of cellular state that resulted in rapidly decreasing of light emission (Węgrzyn and Czyż, 2002).

N) *T-test* was conducted on the solids content data and peak undrained shear strength data to determine the significance of the difference between experimental columns and control columns. t-test was performed A t-test is a statistical test that is used to compare the means of two groups.

4 RESULTS AND DISCUSSION

4.1 Task 1: small-scale column testing for environmental parameters

Table 4.1 shows an overview of the small-scale column tests for FFT. These were all performed at room temperature. Initial sediment concentration, initial S_c, initial sediment thickness, and whether worms were added to the tailings or not, are the variables indicated in the table. In the experiments with worms, a total of 39 *Tubifex* individuals were added. This is equivalent to a concentration of 5000 individuals/m^2, which is consistent with field observations in fresh water bodies in Europe (de Lucas Pardo et al., 2014). The worms concentration can also be given in volumetric units, being approximately 16.5×10^3 individuals/m^3. Moreover, the 39 *Tubifex* individuals weighted 1.2 g, resulting therefore in a concentration of 500g of worms per m^3 of tailings. The initial sediment concentration in columns FFT 1 and FFT 2 is smaller than typical values of gelling concentration (i.e. the concentration at which a bed begins to exist) of FFT. This is therefore an important difference with respect to all other columns, as these two columns did first undergo settling until a bed was created, upon which dewatering started. For the rest of the columns, the test starts with a bed already formed, and dewatering occurs since the beginning. FFT 1 and FFT 2 results are used to calculate the gelling concentration of FFT, with FFT 3 being used for verifying the obtained gelling concentration. FFT 4, FFT 5, and FFT 6 are used to assess the effect of worms in dewatering of in-situ FFT concentrations.

Test id	**FFT 1**	**FFT 2**	**FFT 3**	**FFT 4**	**FFT 5**	**FFT 6**
Temperature	20°C	20°C	20°C	20°C	20°C	20°C
Initial sediment concentration (g/l)	40	48	180	348	348	348
Initial sediment thickness (cm)	30*	30*	30	30	30	30
Initial SC (%)	3.3	4	15	29	29	29
worms	no	no	no	no	yes	yes

Table 4.1. Overview of small-scale column testing for FFT at room temperature in Task1.

Table 4.2 and Table 4.3 show, respectively, an overview of the small-scale column tests for TT at room temperature and in a colder environment. TT 1 and TT 2 are meant to provide a quantification of the gelling concentration of TT, with FFT 3 being used for verifying the obtained gelling concentration. TT 4, TT 5 and TT 6 provide evidence on the influence of worms in dewatering tailings at typical in-situ concentrations of TT. As for the columns at the colder environment, they are all meant to address dewatering potential of worms at a in-situ concentration of TT.

Test id	**TT 1**	**TT 2**	**TT 3**	**TT 4**	**TT 5**	**TT 6**
Temperature	20°C	20°C	20°C	20°C	20°C	20°C
Initial sediment concentration (g/l)	94.5	94.5	432	580.5	580.5	580.5
Initial sediment thickness (cm)	30*	30*	30	30	30	30
Initial SC (%)	7	7	32	43	43	43
worms	no	no	no	no	yes	yes

Table 4.2. Overview of small-scale column testing for TT at room temperature in Task1.

Test id	TT 7	TT 8	TT 9	TT 10
Temperature	10°C	10°C	10°C	10°C
Initial concentration (g/l)	580.5	580.5	580.5	580.5
Initial sediment thickness (cm)	30	30	30	30
Initial SC (%)	43	43	43	43
worms	no	no	yes	yes

Table 4.3. Overview of small-scale column testing for FFT at cold temperature in Task1.

As indicated in section 1.1, a separate set of columns was installed for the falling head tests. These are introduced later in the dedicated section to falling head results (section 4.1.4).

Finally, Table 4.4 shows an overview of the experiments that were added to the scope of the project to test the efficiency of *LV* in increasing the SC, and upon the inclusion of *LV* in the scope of the project. It should be noted that the experiments introduced in Table 4.1 to Table 4.3 were executed in October 2017. The experiments in Table 4.4 were however performed in August 2019 (project was on hold after *Tubifex* became illegal and negotiations with IOSI on the extension of scope took place). By then, tailings might have aged substantially. Therefore we also added reference columns (FFT 7 and TT 11) with no worms on it. Moreover, the *Tubifex* tests were once again repeated (FFT 8 and FFT 12) to compare performance with the results from 2 years earlier. Finally, note that only SC was monitored for the experiments in Table 4.4, and as this was an extra addition to the original scope for which we did not have funding.

Test id	FFT 7	FFT 8	FFT 9	TT 11	TT 12	TT 13
Temperature	20°C	20°C	20°C	20°C	20°C	20°C
Initial sediment concentration (g/l)	348	348	348	580.5	580.5	580.5
Initial sediment thickness (cm)	30	30	30	30	30	30
Initial SC (%)	29	29	29	43	43	43
worms	no	*Tubifex*	*LV*	no	*Tubifex*	*LV*

Table 4.4. Overview of extra small-scale column testing for FFT and TT to test the efficiency of *LV* worms. Initial conditions are equivalent to these in Table 4.1 to Table 4.3. Reference with no worms and tests with *Tubifex* were repeated to position the *LV* results.

4.1.1 Sediment characterization

Upon arrival of the tailings to our laboratories, a number of sediment parameters were studied. These are summarized in Table 4.5. Gelling concentration C_{gel} values were obtained via the dedicated columns indicated in Table 4.1 and Table 4.2. The reported values comply with our expectations on TT and FFT. D_{50} and SFR were obtained via the granulometry of the tailings. Figure 4.1 shows the granulometry of TT and FFT.

	TT	FFT
S_c (%)	43 %	29 %
ρ_{bulk} (kg/m^3)	1350	1200
D_{50} (µm)	17	7,8
SFR (-)	0.42	0.12
C_{gel} (g/l)	165	85
C_{gel} (% of S_c)	12	7

Table 4.5. Results from sediment characterization upon arrival of tailings

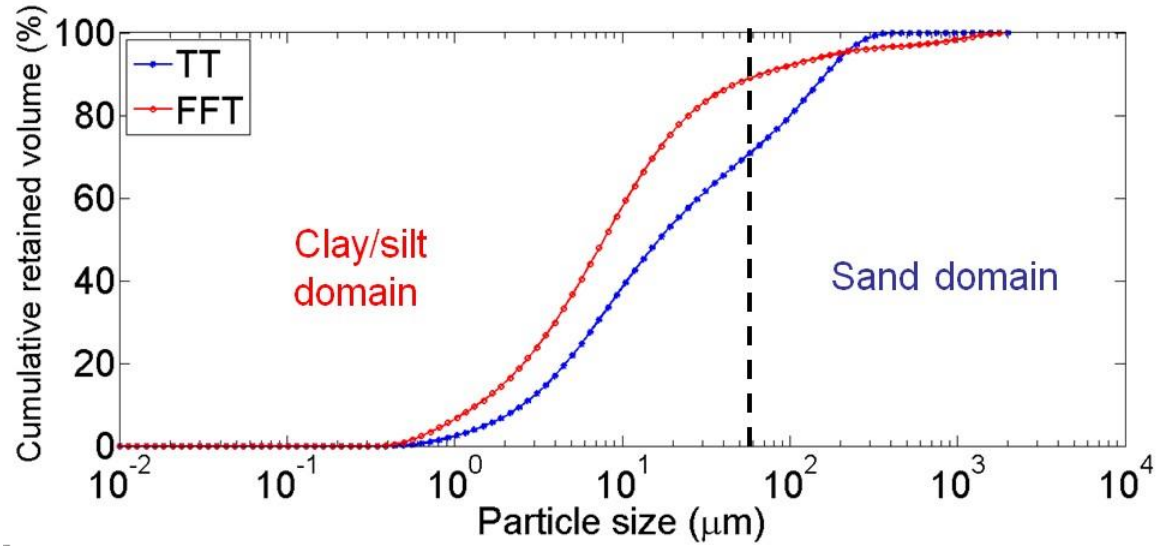

Figure 4.1. Particle size distribution of TT and FFT, as obtained via measurements with a Malvern Mastersizer 2000.

4.1.2 Solids content evolution

A) *Tubifex* in FFT

Figure 4.2 provides a comparison between the performance of column FFT 5 and FFT 6, where 39 *Tubifex* individuals were added (equivalent to 5000 individuals/m^2, or to 16.5 10^3 individuals/m^3 of tailings, or to 500 g of worms/m^3 of tailings), with the performance of column FFT 4 (which did not have any worms in it). The initial conditions of these three columns were identical, with the exception of the presence of worms. The upper panel in Figure 4.2 shows the evolution of the water-bed interface as a function of time, whereas the lower panel shows the evolution of S_c. For the reader's reference, please note that 4 10^4 minutes is approximately a month, and thus that 3 months have passed at 12 10^4 min, with a final experiment time of 3.5 months. For the studied initial conditions ($S_{c,0}$=29%, h_0=30cm), the increase in S_c after 3.5 months is a factor 2 larger for the *Tubifex* treated tailings (from 29% to 37% for *Tubifex*, thus a increase in S_c of 8%; from 29% to 33% for self-weight consolidation only, thus a increase in S_c of 4%). Note also that the largest difference between the dewatering rates with and without worms occurs over the first 1 to 1.5 months (4 10^4 to 6 10^4), with the dewatering rate thereafter being equivalent with and without worms.

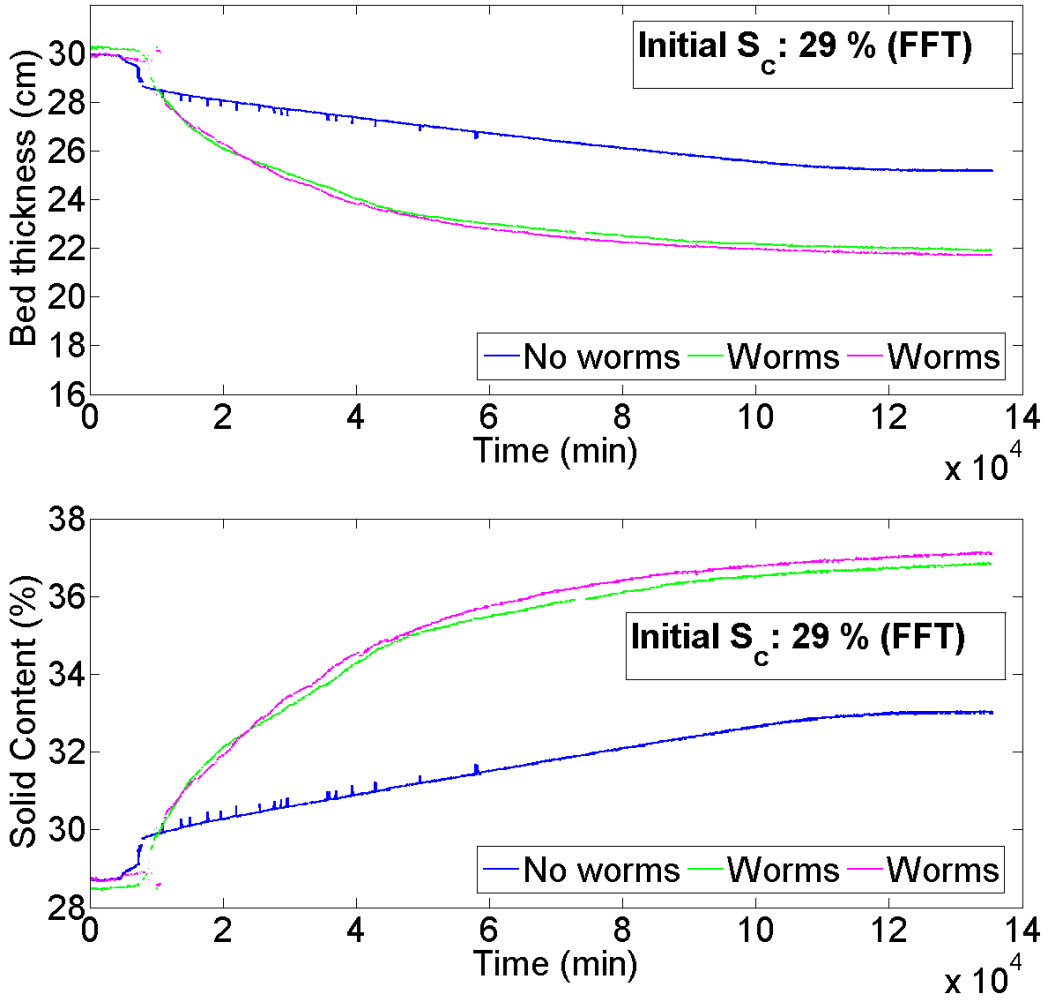

Figure 4.2. Upper panel: Water-bed interface as a function of experiment time. Lower panel: S_c evolution as a function of time. Both panels constitute a comparison between columns FFT 4 (with no worms), and columns FFT 5 and FFT 6 (with worms). 4 x 10^4 minutes is approximately a month, and thus the total experiment time is 3.5 months

B) *Tubifex* in TT

Figure 4.3 provides a comparison between the performance of column TT 5 and TT 6, where 39 *Tubifex* individuals were added (equivalent to 5000 individuals/m^2, or to 16.5 10^3 individuals/m^3 of tailings, or to 500 g of worms/m^3 of tailings), with the performance of column TT 4. Again, the initial conditions of these three columns were identical, with the exception of the presence of worms. The upper panel in Figure 4.3 shows the evolution of the water-bed interface as a function of time, whereas the lower panel shows the evolution of S_c. For the studied initial conditions ($S_{c,0}$=43%, h_0=30cm), the increase in S_c after 3 months is again a factor 2 larger for the *Tubifex* treated tailings (from 43% to 60% for *Tubifex*, thus a increase in S_c of 17%; from 43% to 51% for self-weight consolidation only, thus a increase in S_c of 8%). As in the case of FFT, the latter occurs during the first 1.5 months of experiment.

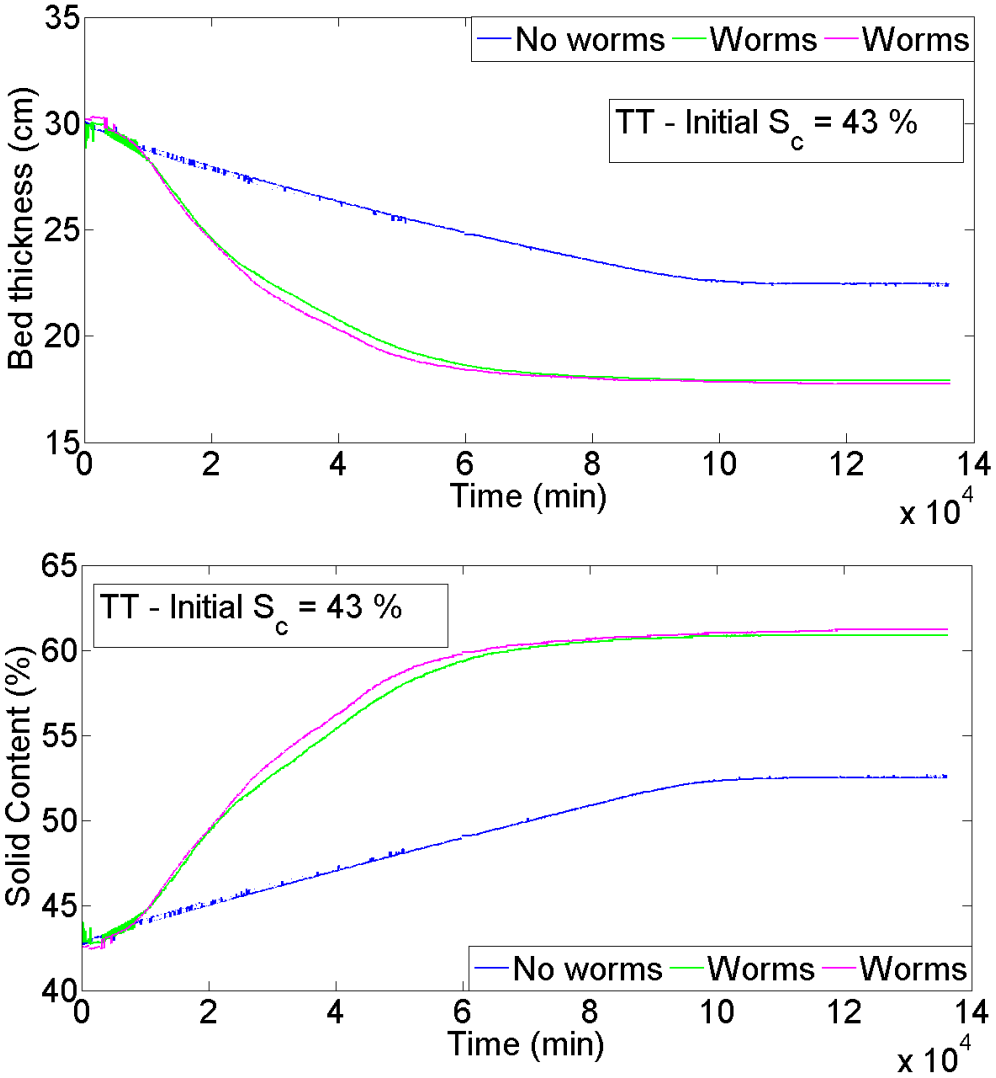

Figure 4.3. Upper panel: Water-bed interface as a function of experiment time. Lower panel: S_c evolution as a function of time. Both panels constitute a comparison between columns TT 4 (with no worms), and columns TT 5 and TT 6 (with worms). 4 x 10^4 minutes is approximately a month, and thus the total experiment time is 3.5 months

C) *Tubifex* in colder temperatures (10 °C); results for TT only

Finally, Figure 4.4 provides a comparison between the performance of column TT 9 and TT 10, where 39 *Tubifex* individuals were added (equivalent to 5000 individuals/m^2, or to 16.5 10^3 individuals/m^3 of tailings, or to 500 g of worms/m^3 of tailings), with the performance of columns TT 7 and TT 8. The initial conditions of these four columns were identical, with the exception of the presence of worms. These results were obtained at 10 °C, whereas the results in Figure 4.3 were obtained for 20 °C. Also, the duration of this test was smaller than these in Figure 4.2 and Figure 4.3, finalizing at 10 10^4 or 2.5 months. For the studied initial conditions ($S_{c,0}$=43%, h_0=30cm), the increase in S_c after 3 months is again a factor 2 larger for the *Tubifex* treated tailings (from 43% to 57% (average of the two tests) for *Tubifex*, thus an increase in S_c of 14%; from 43% to 50% for self-weight consolidation only, thus an increase in S_c of 7%;). Despite the consistency with respect to the increase in S_c , there are two relevant differences between the results shown in Figure 4.4 and the results shown earlier in this section.

The first difference is that for the first time we have observed varying results for the worms treatment, with the two curves not following each other closely as in Figure 4.2 and Figure 4.3. This is in fact very normal, and the reason why biological tests are in duplicates in this study, even in triplicates in standard biology research. For this particular case, we hypothesize that the lower temperature limited the activity of the worms (Davis, 1974; de Lucas Pardo, 2013), which constituted yet a new disadvantage when dealing with the anyway aggressive environment that the tailings are for the worms, ultimately resulting in one of the tested worm populations performing under average.

The second difference is that the largest dewatering rates observed for these tests do not occur over the first 1.5 months as for the FFT and the TT columns at room temperature (see Figure 4.2 and Figure 4.3). In this case, the largest dewatering rates were observed between 4 10^4 and 8 10^4, thus over the second month of testing. This is probably because the worms need more time to adapt to the new environment under their lower mobility due to the lower temperature, and only manage to deliver their optimal dewatering rates (which must be linked to their optimal tunneling rates) after an adaptation period.

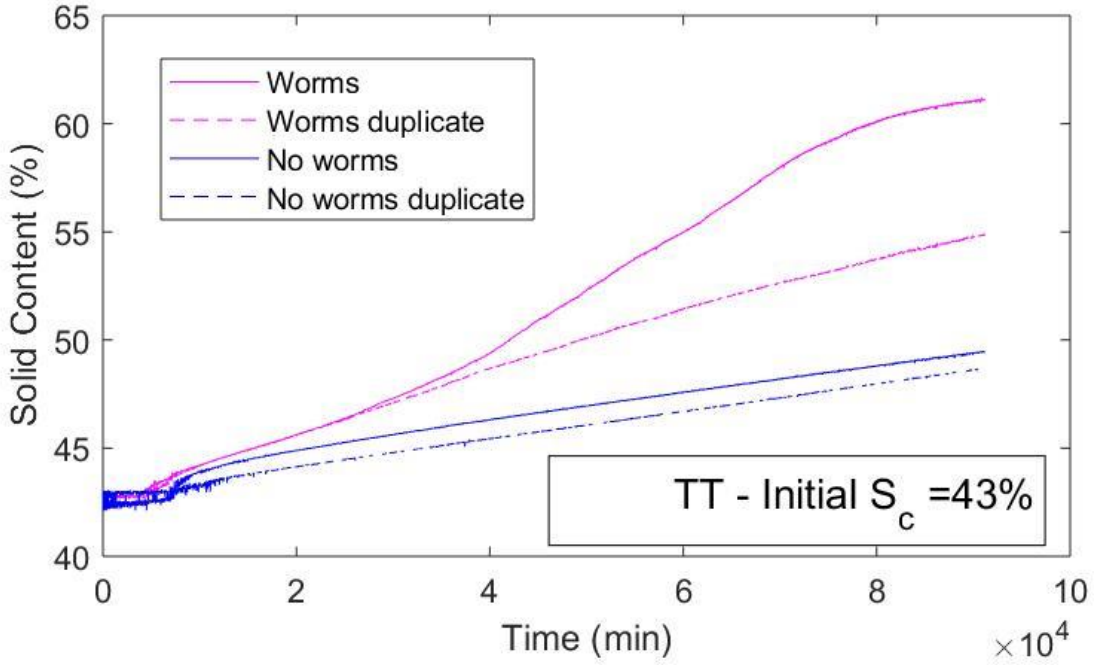

Figure 4.4. S_c evolution as a function of time for columns TT 7 and TT 8 (with no worms), and columns TT 9 and TT 10 (with worms). 4 x 10^4 minutes is approximately a month, and thus the total experiment time is 2.5 months

C) *LV* in FFT

Figure 4.5 provides a comparison between the performance of column FFT 9, where 39 *LV* individuals were added (equivalent to 5000 individuals/m^2, or to 16.5 10^3 individuals/m^3 of tailings, or to 500 g of worms/m^3 of tailings), with the performance of columns FFT 8, with 39 *Tubifex* individuals, and with the performance of FFT 7 (with no worms). The initial conditions of these three columns were identical, with the exception of the presence and the type of worms. Experiments with FFT and no treatment and FFT and *Tubifex* are in fact a repetition of these in Figure 4.2. These were repeated because almost 2 years passed between Figure 4.2 and Figure

4.5, and thus the behavior of tailings may have changed because of aging. The results of the *Tubifex* treated column are consistent with these in Figure 4.2, in fact slightly improving them (37% Sc in Figure 4.2, 38% here). As for the reference with no worms, the final Sc is also equivalent to this in Figure 4.2. What changes is the slope of the curve, which was asymptotical to a horizontal line in Figure 4.2, but a steadily increasing line now. This effect is less visible in the data from the worm treated samples. This is attributed to aging of the tailings, which made even slower in dewatering and consolidating. As for the *LV* performance, the increase in S_c after 3 months is a factor 1.75 larger for the *LV* treated tailings (from 29% to 36% for *LV*, thus an increase in S_c of 7%; from 29% to 33% for self-weight consolidation only, thus an increase in S_c of 4%). This constitutes a smaller increase than in the case of *Tubifex*, where a factor 2 increase in S_c was measured, but remains a relevant improvement of dewatering performance.

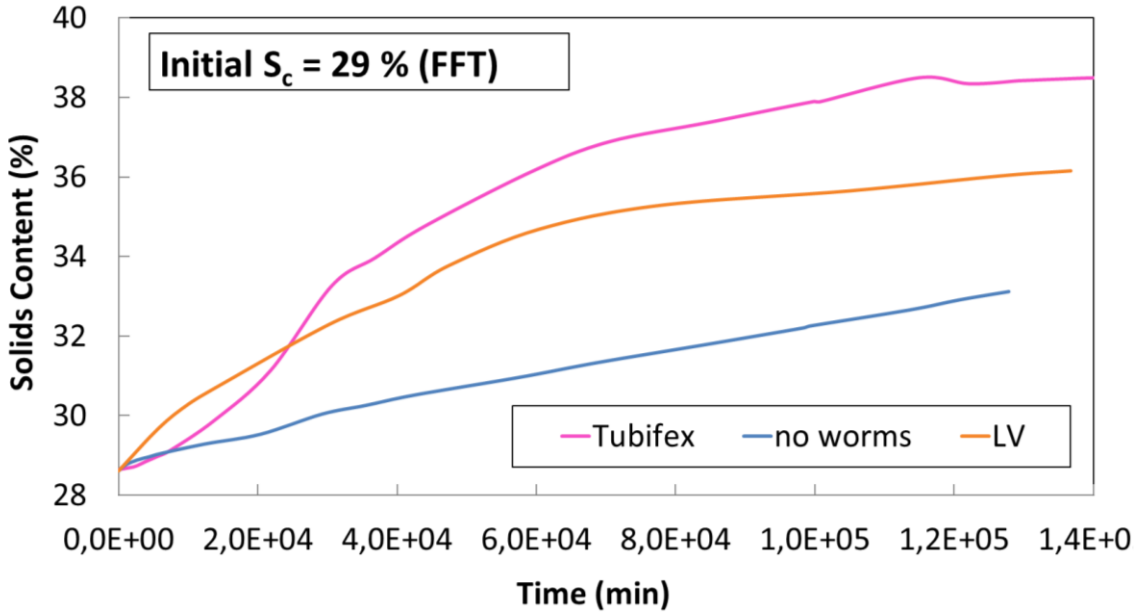

Figure 4.5. S_c evolution as a function of time. These results constitute a comparison between columns FFT 7 (with no worms), FFT 8 (with *Tubifex*) and FFT 9 (with *LV*). 4 x 10^4 minutes is approximately a month, and thus the total experiment time is 3.5 months

D) *LV* in TT

Finally, Figure 4.6 provides a comparison between the performance of column TT 13, where 39 *LV* individuals were added (equivalent to 5000 individuals/m^2, or to 16.5 10^3 individuals/m^3 of tailings, or to 500 g of worms/m^3 of tailings), with the performance of columns TT 12, with 39 *Tubifex* individuals, and with the performance of TT 11 (with no worms). The initial conditions of these three columns were identical, with the exception of the presence and the type of worms. Experiments with TT and no treatment and TT and *Tubifex* are in fact a repetition of these in Figure 4.3. These were repeated because almost 2 years passed between Figure 4.3 and Figure 4.6, and thus the behavior of tailings may have changed because of aging. The final S_c of the *Tubifex* treated column is consistent but slightly smaller than these in Figure 4.3 (60% S_c in Figure 4.2, 57% here, resulting in a relative increase in Sc that is a factor 1.5 larger than in the absence of worms, instead of the factor 2 found in Figure 4.3). As for the reference with no worms, the final S_c is also equivalent to this in Figure 4.3. As in the case of FFT, what changes is the slope of the curve, which was asymptotical to a horizontal line in Figure 4.3, but a steadily increasing line now. As for the *LV* performance, the increase in S_c after 3 months is only a factor

1.33 larger for the *LV* treated tailings (from 43% to 55% for *LV*, thus a increase in S_c of 14%; from 43% to 52% for self-weight consolidation only, thus a increase in S_c of 9%). This constitutes a smaller increase than in the case of *Tubifex*, where a factor 1.5 increase in S_c is measured, but remains a relevant improvement of dewatering performance. In general, the shape of the curves in Figure 4.6 depicts tailings that dewater slower than 2 years before (thus than in Figure 4.3). This effect is visible also for worm treated beds, unlike in the case of FFT where only the non-treated samples had a slow dewatering.

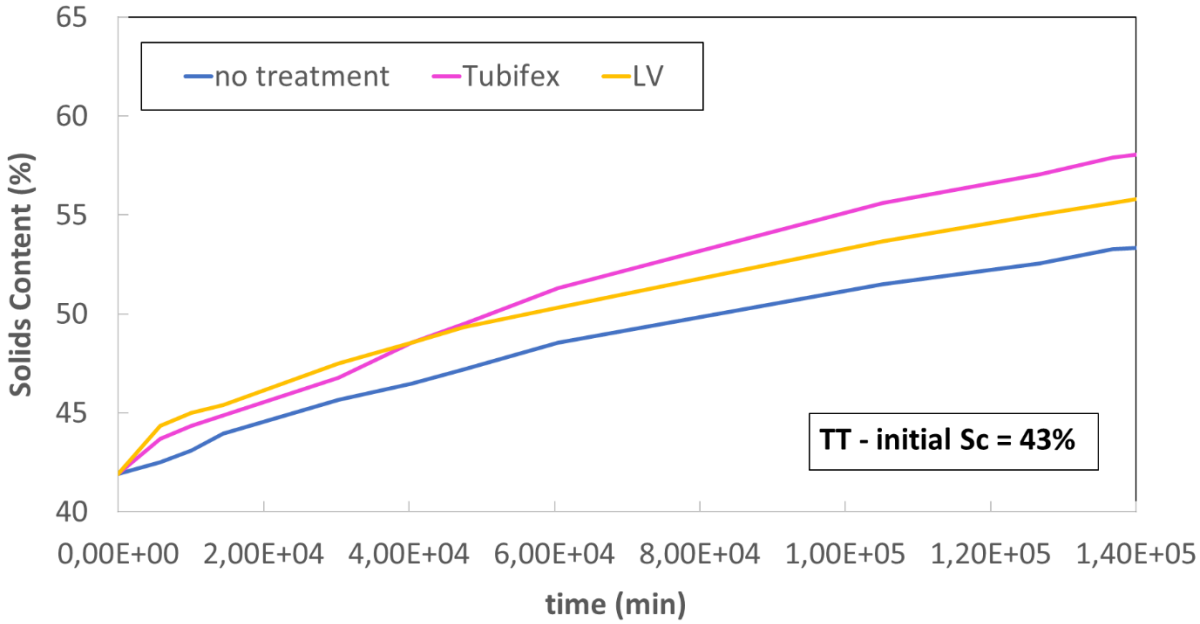

Figure 4.6. S_c evolution as a function of time. These results constitute a comparison between columns TT 11 (with no worms), TT 12 (with *Tubifex*) and TT 13 (with *LV*). 4 x 10^4 minutes is approximately a month, and thus the total experiment time is 3.5 months

4.1.3 Tailings properties evolution

A) Strength

Figure 4.7 shows the undrained peak (upper panel) and remoulded (lower panel) shear strength measured after 3.5 months consolidation in columns FFT 4, FFT 5 (worms) and FFT 6 (worms). The vertical grey dotted line represents the undrained peak shear strength upon arrival of the tailings. Both the peak and remoulded strength measurements show larger strengths at all depths than in the absence of worms. At the surface, strengths are typically a factor 2 to 2.5 larger, which decreases to less than a factor 1.5 at 11 cm deep. In some of the measurements a malfunction of the vane resulted in the absence of data (e.g. FFT warm room worms dup a -6 cm depth).

Figure 4.8 shows the undrained peak (upper panel) and remoulded (lower panel) shear strength measured after 3.5 months consolidation in columns TT 4, TT 5 (worms) and TT 6 (worms). For these tailings worms resulted in a factor 1.5 larger undrained peak shear strength and a factor 2.5 larger for undrained remoulded shear strength. The strength results for TT showed profiles with a less pronounced gradients towards the bottom of the columns.

Finally, Figure 4.9 shows the undrained peak (upper panel) and remoulded (lower panel) shear strength measured after 3.5 months consolidation in columns TT 7, TT 8 (worms) and TT 9 (worms), this time for a temperature of 10 °C (all other results were obtained for 24 °C). At this

temperature worms resulted in almost a factor 3 larger undrained peak shear strength at a depth of -1 cm, which decreases gradually over depth. Also, the strength of TT samples with no worms ranged between 40 and 120 Pa. For room temperature larger strengths were found, ranging from 100 to 120 Pa. The worms treated TT experiment also resulted in larger strengths in cold (160 to 180 Pa at 24 °C, with 120 to 160 Pa at 10 °C), but the differences were less pronounced than in the case of tailings only.

As worms tend to result in the largest increase of strength (relative to this in the absence of worms) at the surface, the strength profiles in worm treated beds exhibit a smaller vertical gradient over depth, to the point of causing inverted strength profiles in some cases. Moreover, it is worth mentioning that the strengths registered by a worm treated FFT bed (100 to 120 Pa) are in the same range as these of consolidated TT with no worms (also 100 to 120 Pa). Thus it can be stated that 30 cm layers of FFT that were treated with worms are as strong as 30 cm layers of TT after consolidation.

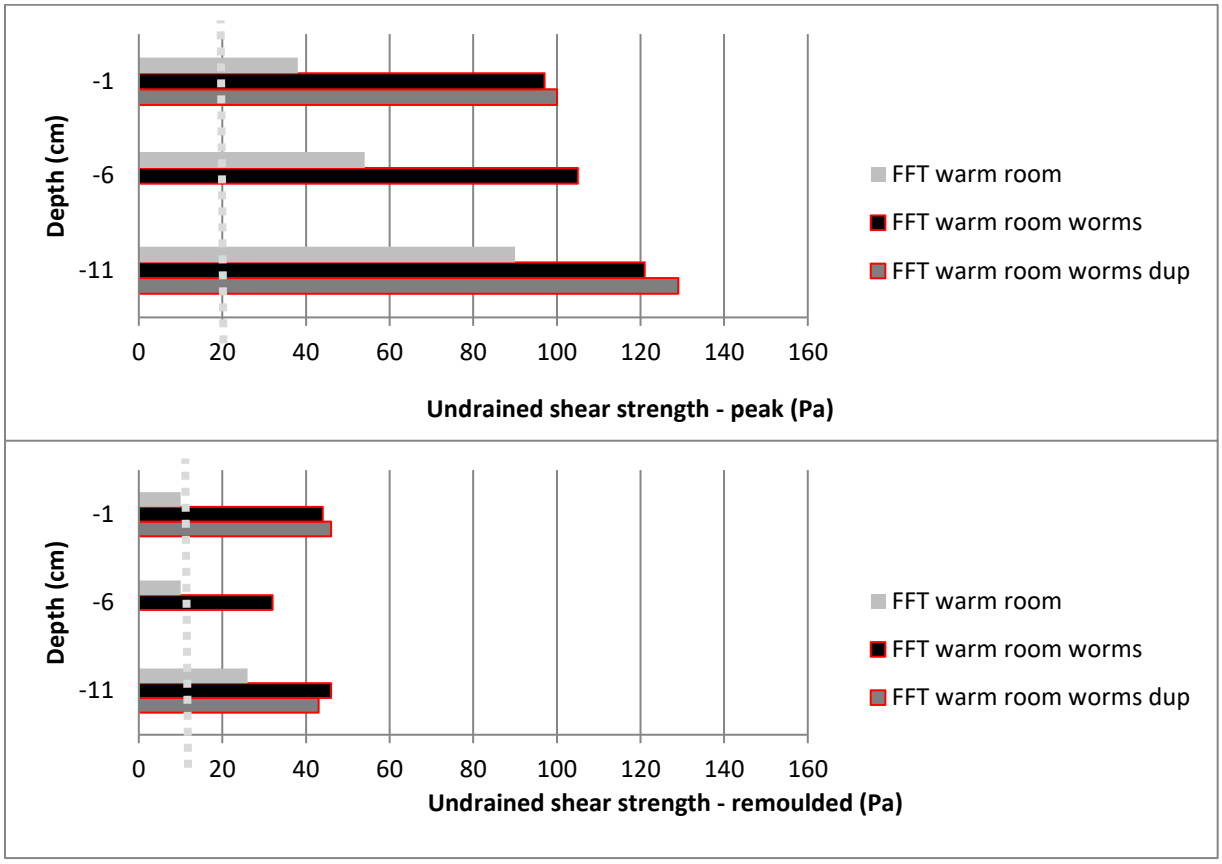

Figure 4.7. Undrained peak (upper panel) and remoulded (lower panel) shear strength measured after 3.5 months consolidation in columns FFT 4, FFT 5 (worms) and FFT 6 (worms). The vertical grey dotted line represents the undrained peak shear strength upon arrival of the tailings.

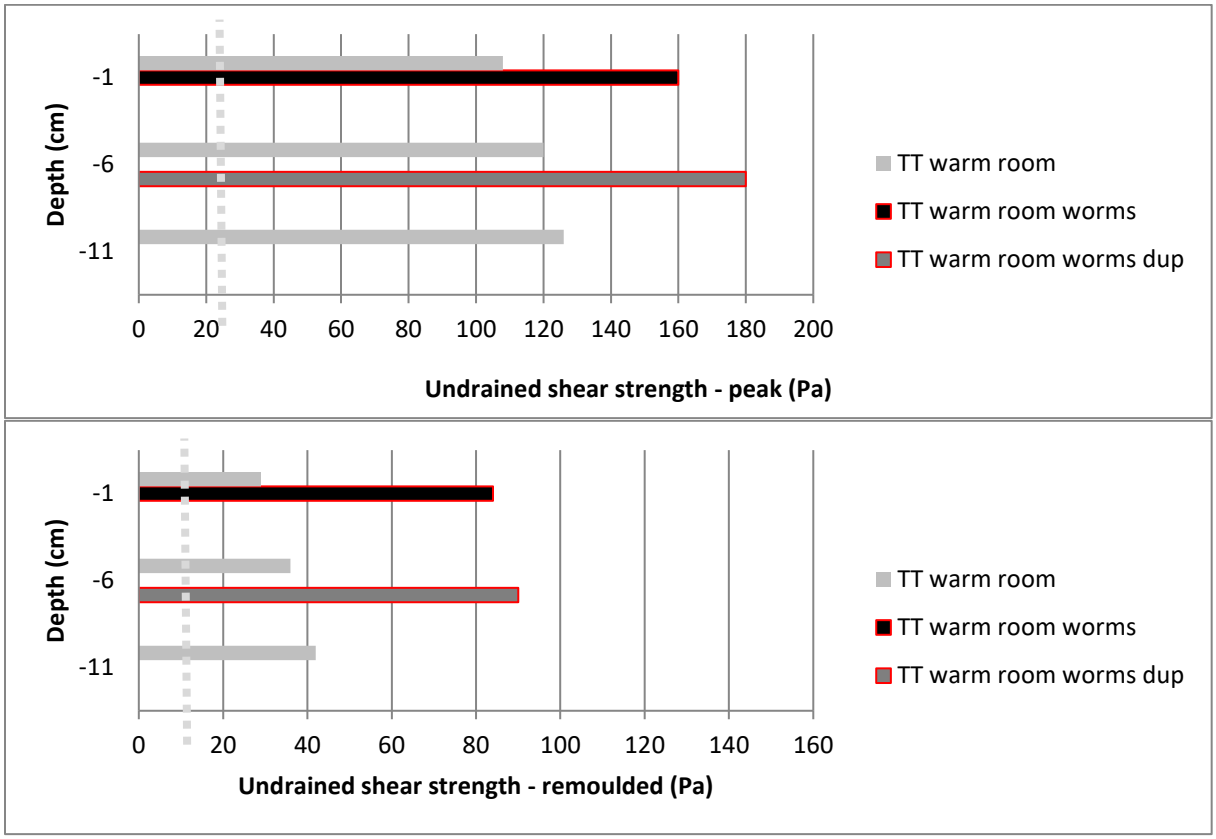

Figure 4.8. Undrained peak (upper panel) and remoulded (lower panel) shear strength measured after 3.5 months consolidation in columns TT 4, TT 5 (worms) and TT 6 (worms). The vertical grey dotted line represents the undrained peak shear strength upon arrival of the tailings.

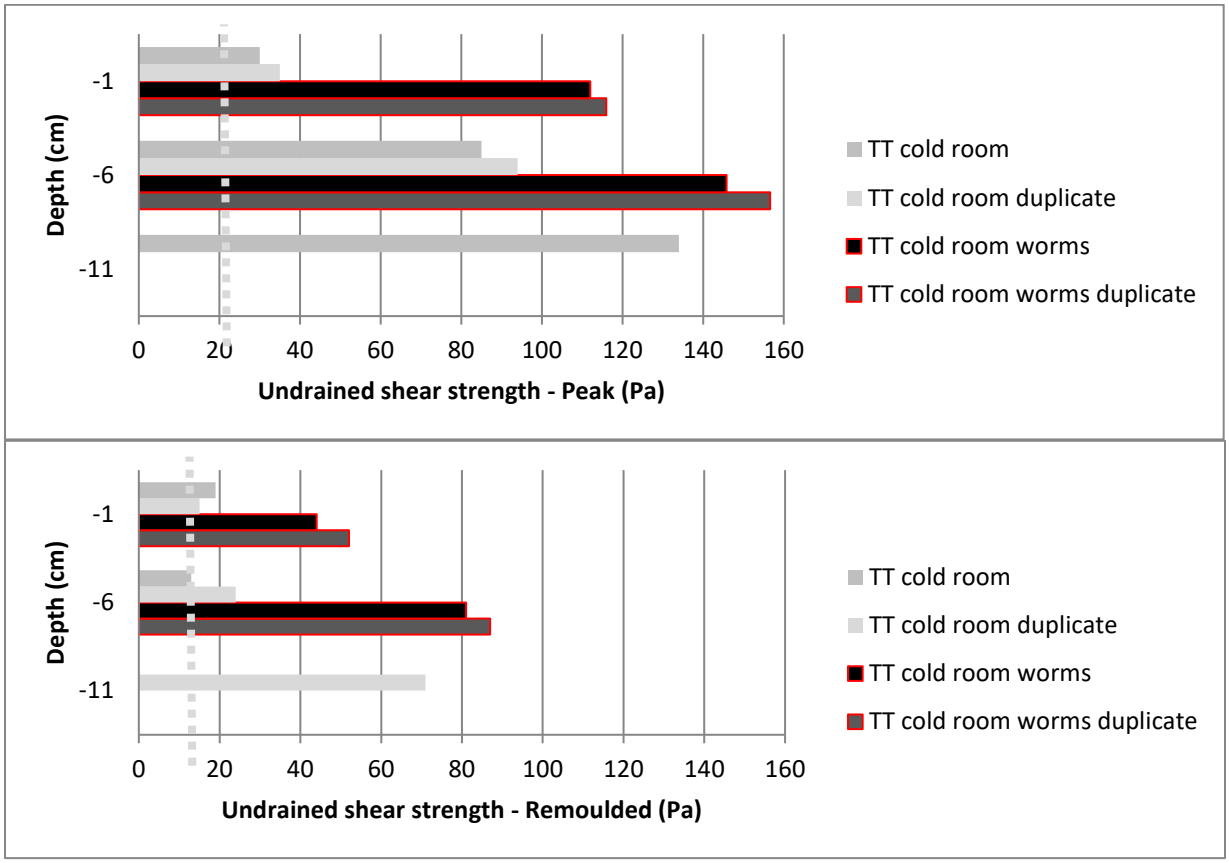

Figure 4.9. Undrained peak (upper panel) and remoulded (lower panel) shear strength measured after 2.5 months consolidation in columns TT 7, TT 8, TT 9 (worms) and TT 10 (worms). The vertical grey dotted line represents the undrained peak shear strength upon arrival of the tailings.

B) Density

Figure 4.10 to Figure 4.12, show bulk density profiles in the exact same order as the strength results in the previous section were presented: FFT, TT, and TT at a lower temperature. In general worms treated beds exhibit a 100 kg/m^3 density larger than in their absence at all depths. In the case of lower temperature, this difference is larger at the surface (200 kg/m^3) and smaller at the bottom of the columns (approximately 50 kg/m^3 in average). Unlike in the case of strength, the bulk density of a FFT worm treated bed does exceeds this of a consolidated layer of TT. Nevertheless, the bulk density of a FFT worm treated bed (1400 to 1500 km/m^3) is larger than the initial bulk density of TT (1350 kg/m^3), which can remain interesting.

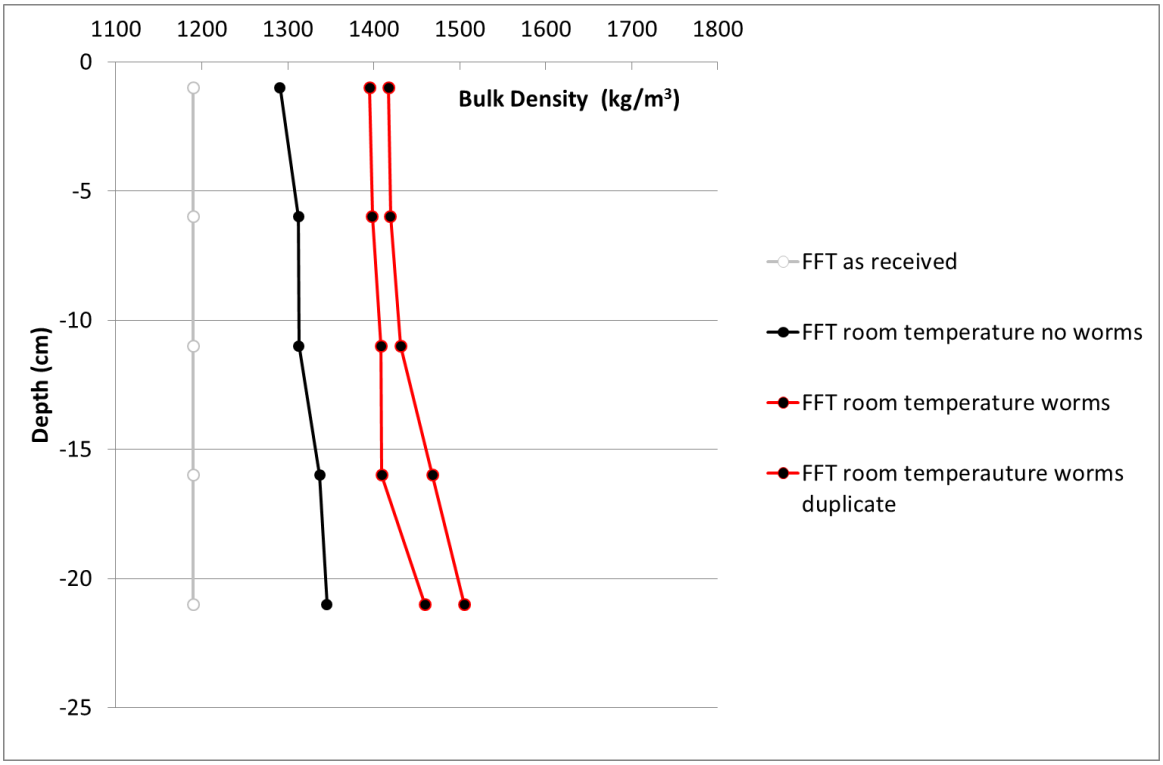

Figure 4.10. Bulk density measurements after 3.5 months consolidation in columns FFT 4, FFT 5 (worms) and FFT 6 (worms). The vertical grey line represents the bulk density upon arrival of tailings.

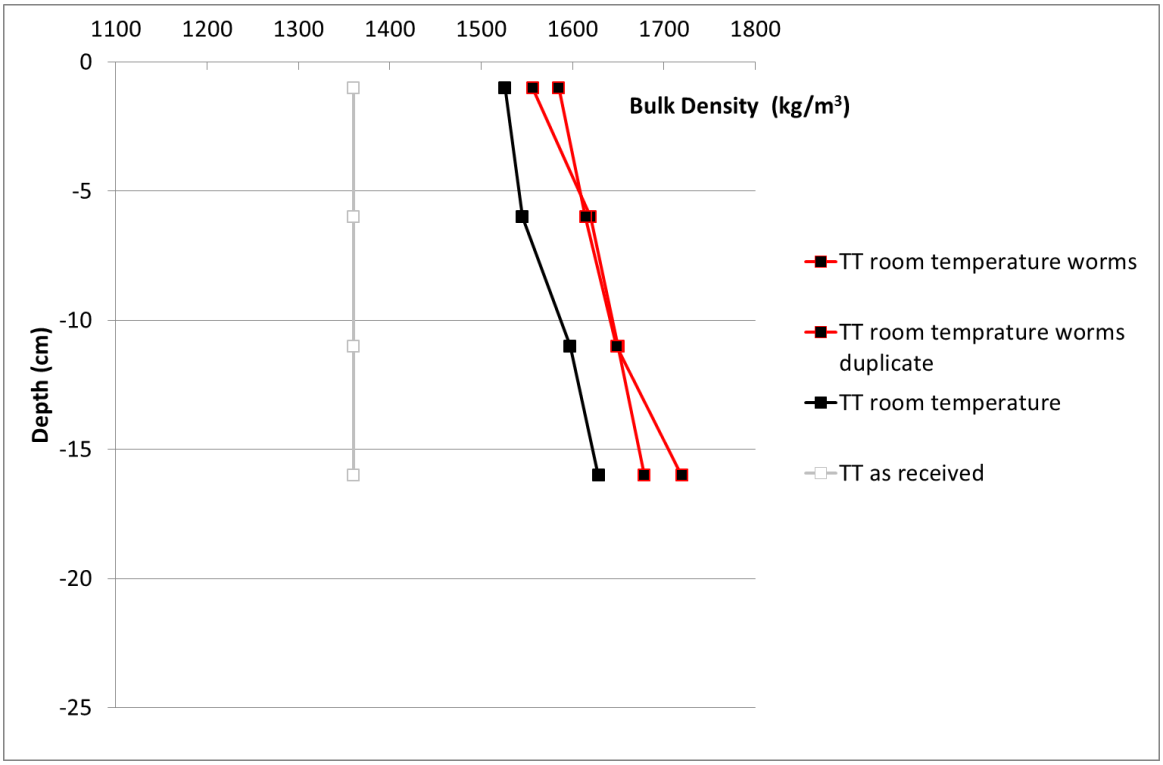

Figure 4.11. Bulk density profiles measured after 3.5 months consolidation in columns TT 4, TT 5 (worms) and TT 6 (worms). The vertical grey line represents the bulk density upon arrival of tailings.

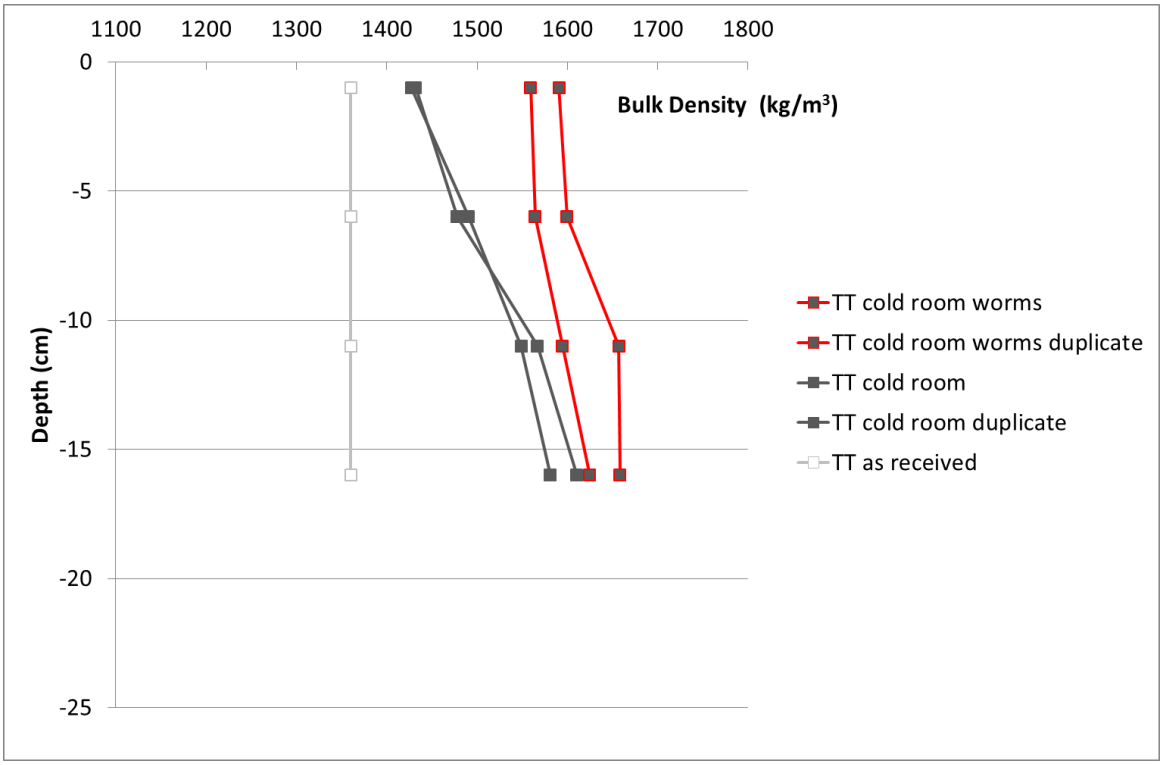

Figure 4.12. Bulk density profiles measured after 2.5 months consolidation in columns TT 7, TT 8, TT 9 (worms) and TT 10 (worms). The vertical grey line represents the bulk density upon arrival of tailings.

C) Plasticity

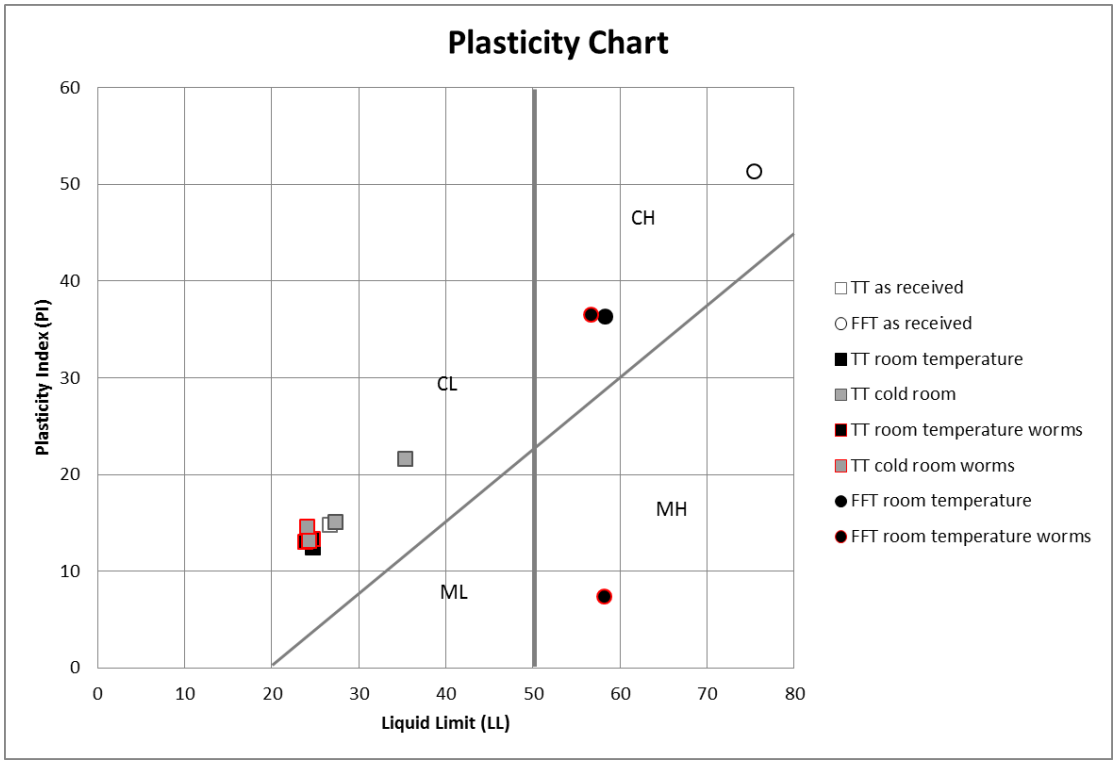

Figure 4.13. Chart of Atterberg limits relative to plasticity index. Square markers represent TT, and circular markers represent FFT. The markers with no fill represent Atterberg limits of tailings as received. Markers with any fill represent Atterberg Limits after the consolidation tests took place. The markers with black fill represent tests performed at room temperature (24 °C). The markers with grey fill represent test at the cold temperature (10 °C). Finally, markers surrounded by a red line indicate tests where worms where added. ML: low plasticity silt; MH: high plasticity silt; CL: low plasticity clay; CH: high plasticity clay;

According to the results from the Atterberg limits as presented in Figure 4.13, TT as received can be classified as low plasticity clay, with FFT as received being high plasticity clay. For TT, all columns stay within the same domain (low plasticity clay) after having consolidated. Only one TT column exhibits a somehow different LL and PI after consolidation (one of the TT cold room columns). As for FFT, there is one FFT column that becomes classified as a high plasticity silt after consolidation. We do not have an explanation for this measurement. The other two columns exhibited a consistent (with each other) decrease of LL and PI following the slope of the A-line, but do remain within the high plasticity clay domain.

Overall, it can be concluded that the plasticity of TT columns is in general not modified after self weight consolidation and after worms treatment. The more limited data set collected for FFT indicates that FFT samples decrease in plasticity upon consolidation, but independently of the treatment with worms.

4.1.4 Falling head tests: permeability

Table 3.1 gives an overview of the falling head tests. The initial concentrations are the concentrations of the tailings as received, and coincide with the relevant initial concentrations

tested for their S_c evolution (see section 4.1.2). The studied tailings thickness is also consistent with dewatering tests. The initial water height above the bed was always set to 1 m.
The worms are added according to the natural density 5000 worms/m^2 (or 16.5 10^3 individuals/m^3 of tailings, or to 500 g of worms/m^3 of tailings), 88 individuals for the larger cross section of the falling head tests. The worms are let to re-work the soil matrix for 5 weeks. After 5 weeks the soil layer in all columns consolidated but in columns with worms the compaction rate is higher (again consistently with the findings of section 4.1.2). The experiment starts by opening the hose to column 2 (see again Figure 3.3) and measuring Δh_t directly. The bed height and head differences are recorded daily for four weeks thereafter weekly for three weeks more. Columns were covered with foil to avoid evaporation (which we acknowledge may have had a negative impact in further re-working by the worms; but a correct water balance is crucial to obtain results in this test).

Test id	FFT 1_fh	FFT 2_fh	TT 1_fh	TT 2_fh	FFT 3_fh	FFT 4_fh	TT 3_fh	TT 4_fh
initial sediment concentration (g/l)	348	348	580	580	348	348	580	580
Initial bed thickness (cm)	30	30	30	30	30	30	30	30
initial *Sc* (%)	29	29	43	43	29	29	43	43
worms	no	no	no	no	yes	yes	yes	yes

Table 4.6. Overview of falling head tests, including test id, initial concentration and equivalent initial *Sc*, and presence/absence of worms treatment

Figure 4.14 shows the results from the falling head measurements for FFT, both with and without worm treatment. The x-axis represents void ratio (-), and y-axis gives the permeability *K* (m/s) measurements per void ratio as obtained from the falling head tests. At the beginning of the test the bed is less copact and thus we are in the right hand side of the graph, at large void ratios. As consolidation and dewatering goes on (which occurs both in the absence and in the presence of worms; the dewatering rate improves with worms, but the bed becomes compact in the absence of worms too due to self-weight consolidation), the bed becomes more compact and the void ratio decreases (see the definition of void ratio e at section 3.2.4: as dewatering goes on, the volume of water decreases and thus the ratio between the voume of water and the volume of soilds becomes smaller). Green triangles and purple crosses represent measurements to the worm treated beds, with blue diamonds and red squares representing beds without worms. In general, Figure 4.14 shows larger permeabilities for worm treated columns. This means that the worm treated beds do indeed dewater faster, explaining the results in section 4.1.2. Moreover, worm treated beds exhibit a range of variation of void ratios that is smaller than in the absence of worms too, and given the more compact nature of a worm treated bed.

To understand and quantify to which extent does a worm treated bed dewater faster than in the absence of worms, let us look to the void ratios associated to a permeability of 1 10^{-7} m/s. With worms, this is permeability is exhibited for a void ratio of a bit smaller than 5, and in the absence of worms 1 10^{-7} m/s is associated to a void ratio a bit larger than 6. Translating to solids contents, this is approximately 34% with worms and 29% without worms. This means that worms can deliver the same permeability as no worms for a increase of 5% in *Sc*. As the bed continues becoming more compact with time the gap between the void ratio delivering the same permeability decreases (see for example 1 10^{-8}: the void ratio with no worms is 5.2, and with worms is 4.4; the gap in void ratio delivering the same permeability have decreased from 1 unit to 0.8 units). When the permeability approaches 1 10-9 the void ratios of worm and no worm

beds are only separated by 0.4. Nevertheless the data becomes more unclear towards this point too (more frequent jumps in permeability for similar void ratios, which is associated with the vertical resolution of the head measurements as indicated in section 3.2.4), making it difficult to withdraw sound conclusions from this region of the graph.

The other way to look at the results in Figure 4.14 is by looking at the different permeabilities exhibited by a bed of equal void ratio, with and without worms. Let us look at the permeabilities associated to a void ratio of 5 (which is equivalent to a Sc of 35%). A bed treated with worms has a permeability of 1 10-7, whereas a bed with no worms with the exact same void ratio has a permeability of 2 10-09, This is why worms treated beds end up being much more compact than non-treated bed in equilibrium: the worms manage to increase the permeability almost a factor 100 for the same solid content (for a specific range of solid contents of course). If we then look at the permeabilities associated with a void ratio of 4 (39% solid contents), it becomes 2 10-9 for worms and 1 10-09 for no worms, being therefore only a factor 2 larger for worms. Therefore the gap in permeabilities decreases as the void ratio decreases too. Nevertheless, worm treated bed still exhibit a smaller final void ratio (and the associated Sc as reported in section 4.1.2), meaning that even when the permeabilities become equal, worm treated beds remain more compact.

Finally, it is also worth indicating that the largest differences between a worm treated bed and a non-treated one, are exhibited over the first days of experiment. This is also the period where the worms do most of their re-working, as indicated by the results from section 4.1.2.

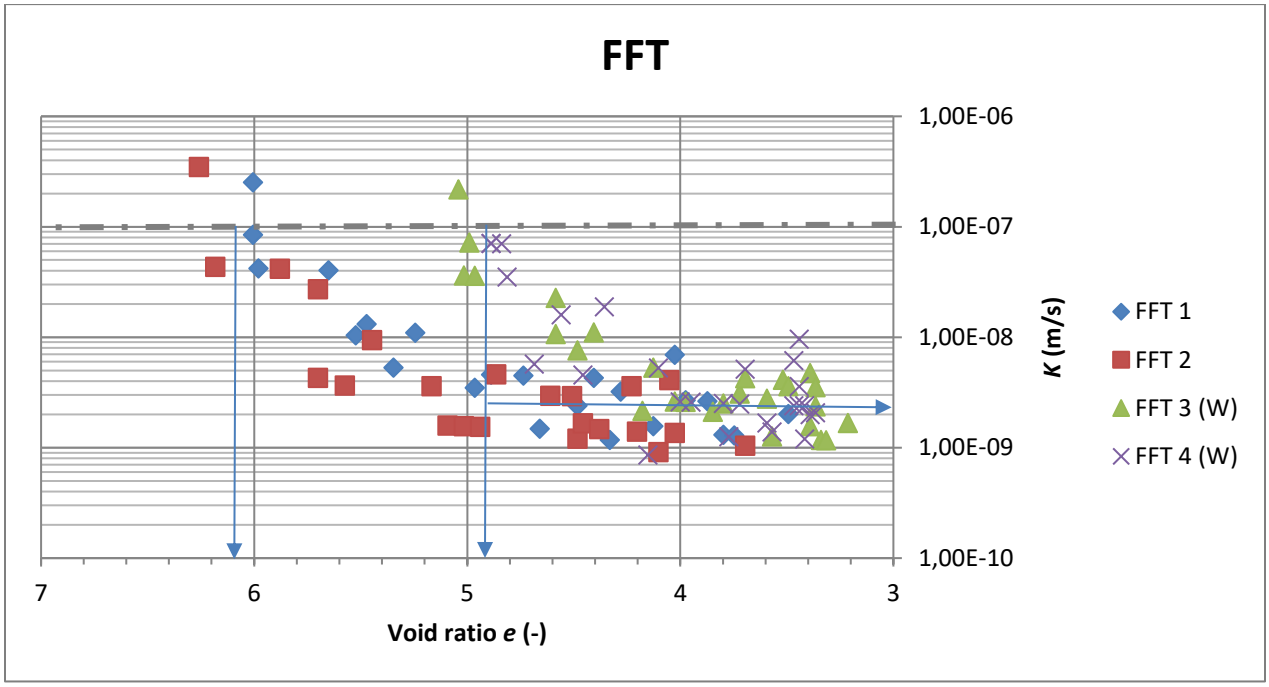

Figure 4.14. Falling head results for FFT, highlighting differences between worms and no worms treatment. The x-axis represents the void ratio (-) of the sediment in the columns. That y-axis is the permeability (m/s) as obtained for the falling head tests.

Figure 4.14 shows the results from the falling head measurements for TT, both with and without worm treatment. These measurements were obtained following the exact same protocol as for the

results obtained for FFT ns shown in Figure 4.15. In this case, the permeabilities at the worm treated beds are crearly larger than these of the non-treated beds only for void ratios larger than 2. For smaller void ratios, it is unclear from the data if there are differences between treatments, as it is rather scattered and the data from different treatments overlaps with each other. The latter is probably caused by the inaccuricies in our method as described in section 3.2.4.

For void ratios larger than 2, the same analysis as performed to the FFT data can be repeated: a permeability of 1 10-8 is exhibited by a bed with a void ratio of almost 2.4 in the absence of worms, and for a void ratio of 2 with worms. These void ratios are equivalent to a *Sc* of 51% and of 56% respectively. Again, the same permeability is delivered for a bed that is 5% larger in *Sc* thanks the tunneling of the worms. If we then look at the permeabilities delivered by beds with a void ratio of 2, we will find the with worms the peremeability is 1 10-8, whereas without worms is 2 10-9, and therefore 10 times smaller than with worms.

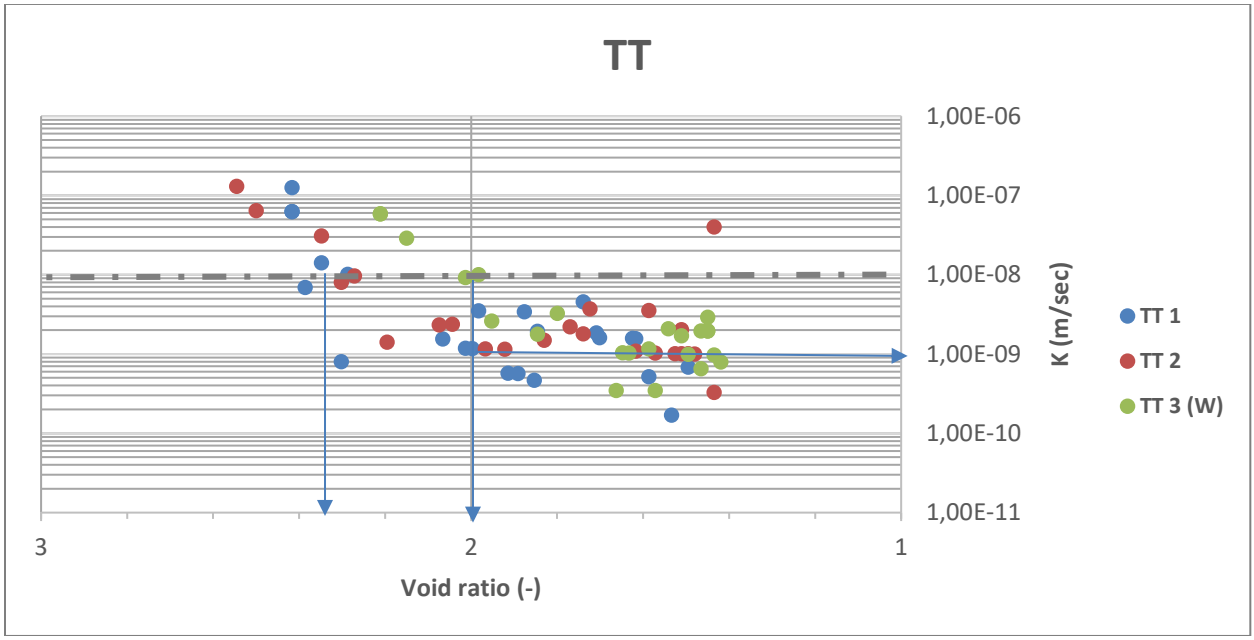

Figure 4.15. Falling head results for TT, highlighting differences between worms and no worms treatment. The x-axis represents the void ratio (-) of the sediment in the columns. That y-axis is the permeability (m/s) as obtained for the falling head tests.

4.2 Task 2: beaker testing for worms survival and reproduction

4.2.1 Task 2A

Table 4.7 gives an overview of the beaker tests performed for studying *Tubifex* survival, including weeks that the test lasted, the type of additive added to the experiment, the test's replicate number, and the date in which the experiment was stopped and worms were counted. In the last column of Table 4.7 the number of worms found alive for each experiment is shown. These results are displayed in Figure 4.16 as well. Results are clear: only LQ om (low quality organic matter) treatment resulted in an increase of *Tubifex* individuals over time. After 3 weeks, all treatments exhibited a decrease in alive *Tubifex* worms. After 6 weeks, the decrease in alive individuals continues for most treatments, whereas LQ om starts displaying an increase. Finally, week 16 reveals a factor 3 reproduction factor (relative to number of worms at $t = 0$) for LQ om, with all other treatments showing either equal or less worms than initially. It is therefore

concluded that what the worms need to be able to reproduce in tailings is LQ om, and that worms need between 6 and 16 weeks time to reproduce in this particular environment (tailings with LQ om).

Other observations can be made in Figure 4.16. First, it should be noted that both the absence of additives and the treatment LQ om + IN c1 display an increase in *Tubifex* numbers between week 6 and week 16. This means likely that there is some sort of minor reproduction going on, as otherwise the decreasing trend should have continued. Worms seem to suffer from an initial shock after being added to tailings (regardless of the additive), but with time reproduction starts to occur and the decreasing trend can be slightly reverted (for LQ om + IN c1), or totally transformed into a increasing trend (LQ om). It is worth noting that in the absence of treatment *Tubifex* reproduction does occur, with worm individuals still having the same numbers as 16 weeks before. This is not consistent with findings from previous projects, which is attributed to some tailings types being less toxic for worms (and in general) than others.

Weeks	worms	additive	replicate number	counting	results
3	*Tubifex*	-	I	29-11-2017	39
3	*Tubifex*	-	II	29-11-2017	34
6	*Tubifex*	-	I	21-12-2017	29
6	*Tubifex*	-	II	21-12-2017	25
16	*Tubifex*	-	I	28-02-2018	36
16	*Tubifex*	-	II	28-02-2018	30
3	*Tubifex*	IN c1	I	29-11-2017	41
3	*Tubifex*	IN c1	II	29-11-2017	38
6	*Tubifex*	IN c1	I	21-12-2017	32
6	*Tubifex*	IN c1	II	21-12-2017	26
16	*Tubifex*	IN c1	I	28-02-2018	25
16	*Tubifex*	IN c1	II	28-02-2018	6
3	*Tubifex*	IN c2	I	29-11-2017	25
3	*Tubifex*	IN c2	II	29-11-2017	27
6	*Tubifex*	IN c2	I	21-12-2017	27
6	*Tubifex*	IN c2	II	21-12-2017	37
16	*Tubifex*	IN c2	I	28-02-2018	24
16	*Tubifex*	IN c2	II	28-02-2018	24
3	*Tubifex*	HQ om	I	29-11-2017	26
3	*Tubifex*	HQ om	II	29-11-2017	40
6	*Tubifex*	HQ om	I	21-12-2017	37
6	*Tubifex*	HQ om	II	21-12-2017	26
16	*Tubifex*	HQ om	I	28-02-2018	23
16	*Tubifex*	HQ om	II	28-02-2018	27
3	*Tubifex*	LQ om	I	29-11-2017	23
3	*Tubifex*	LQ om	II	29-11-2017	27
6	*Tubifex*	LQ om	I	21-12-2017	38
6	*Tubifex*	LQ om	II	21-12-2017	43
16	*Tubifex*	LQ om	I	28-02-2018	97
16	*Tubifex*	LQ om	II	28-02-2018	145

Weeks	worms	additive	replicate number	counting	results
3	*Tubifex*	LQ om + IN c1	I	29-11-2017	31
3	*Tubifex*	LQ om + IN c1	II	29-11-2017	28
6	*Tubifex*	LQ om + IN c1	I	21-12-2017	20
6	*Tubifex*	LQ om + IN c1	II	21-12-2017	22
16	*Tubifex*	LQ om + IN c1	I	28-02-2018	27
16	*Tubifex*	LQ om + IN c1	II	28-02-2018	59

Table 4.7. Overview of treatments to improve *Tubifex* reproduction and survival in FFT. The first column indicates the number of weeks the additive was left to work on the mixture of tailings and worms. The second column indicates the type of worm. Third column shows type of additive (the abbreviations in the legend are introduced in the methodology section, in particular in section 3.3.1). Forth column shows replicate number. Finally, the fifth column shows the date in which the experiment was stopped and worms were counted, with the last column showing the number of worms found alive. These results are also displayed in Figure 4.16.

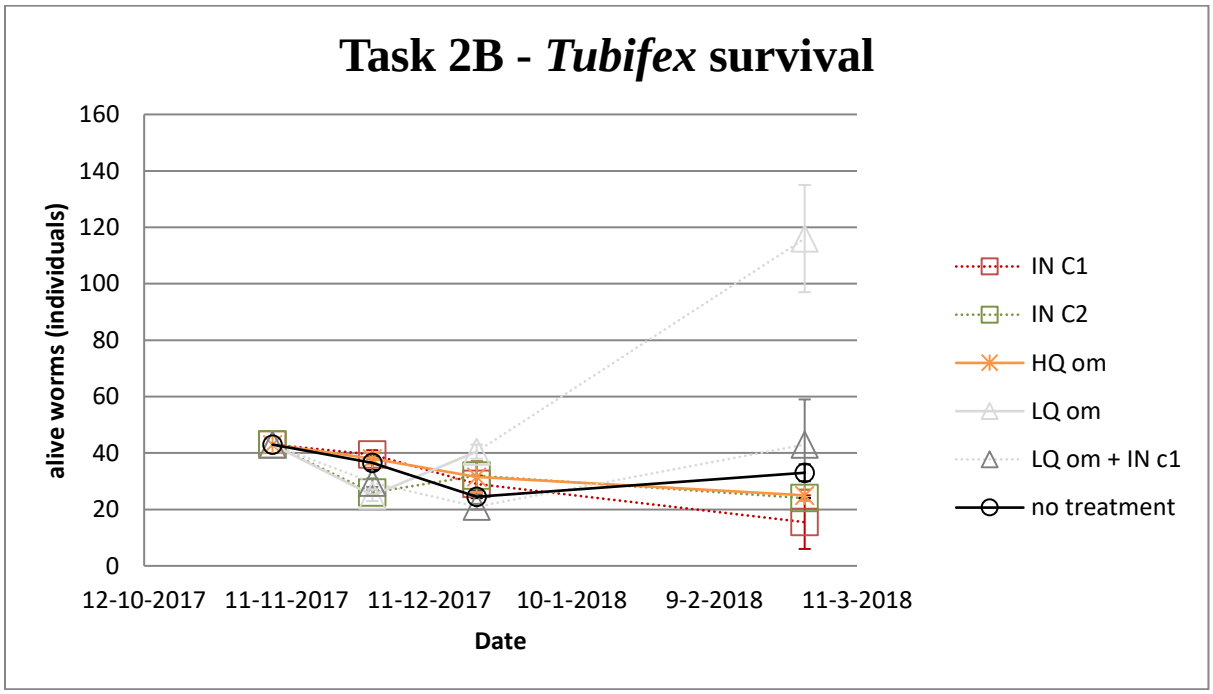

Figure 4.16. Alive *Tubifex* worms as a function of time found for the additives tested in FFT. The abbreviations in the legend are introduced in the methodology section, in particular in section 3.3.1. The error bars account for the variability between duplicates.

4.2.2 Task 2B

Table 4.8 gives an overview of the beaker tests performed for studying *LV* survival, including weeks that the test lasted, the type of additive added to the experiment, the test's replicate number, and the date in which the experiment was stopped and worms were counted. In the last column of Table 4.8 the number of worms found alive for each experiment is shown. These results are displayed in Figure 4.17 as well. As introduced in the methodology section, this time there were more replicates (to have more certainty over the results, and given the intrinsic variability associated to biological processes) and less treatments (we discarded those that did

not work for Tubifex as well, assuming a similar behavior of *Tubifex* and *LV*). Moreover, most tests were stopped and counted at week 16, given the not so relevant changes found for time < 16 weeks in the case of *Tubifex*. We did add few beakers that were meant to be check points at weeks equal 3 and 6, and just to corroborate that the trends as observed in the Tubifex results did still hold (no large variations before week 16). The conclusions remain similar to the *Tubifex* results: LQ om displays the largest increase in *LV* numbers, and also approximately a factor 3 larger than initially. In this case, also LQ om + IN c1 did show a increase in worm individuals or approximately a factor 2. For all other additives and for the absence of them, alive worm individuals decreased as a function of time, but with still approximately half of the initial number of worms alive after 16 weeks. The check point for LQ om + IN c1 at 6 weeks confirms that worms slowly decrease in numbers over the first weeks, with reproduction starting to become relevant somewhere between 6 and 16 weeks.

Weeks	worms	additive	replicate number	counting	results
3	*LV*	-	-	8-1-2019	17
16	*LV*	-	I	4-4-2019	35
16	*LV*	-	II	4-4-2019	35
16	*LV*	-	III	4-4-2019	41
16	*LV*	IN c2	I	4-4-2019	27
16	*LV*	IN c2	II	4-4-2019	15
16	*LV*	IN c2	III	4-4-2019	29
16	*LV*	HQ om	I	4-4-2019	22
16	*LV*	HQ om	II	4-4-2019	22
16	*LV*	HQ om,	III	4-4-2019	23
3	*LV*	LQ om	-	8-1-2019	20
16	*LV*	LQ om	I	4-4-2019	128
16	*LV*	LQ om	II	4-4-2019	107
16	*LV*	LQ om	III	4-4-2019	143
6	*LV*	LQ om + IN c1	-	25-2-2019	14
16	*LV*	LQ om + IN c1	I	4-4-2019	103
16	*LV*	LQ om + IN c1	II	4-4-2019	68
16	*LV*	LQ om + IN c1	III	4-4-2019	77

Table 4.8. Overview of additives to improve *LV* reproduction and survival in FFT. The first column indicates the number of weeks the additive was left to work on the mixture of tailings and worms. The second column indicates the type of worm. Third column shows the date in which the experiment was stopped and worms were counted. Finally, the fourth column shows replicate number, with the last column showing the number of worms found alive. The abbreviations in the legend are introduced in the methodology section, in particular in section 3.3.2. The error bars account for the variability between triplicates.

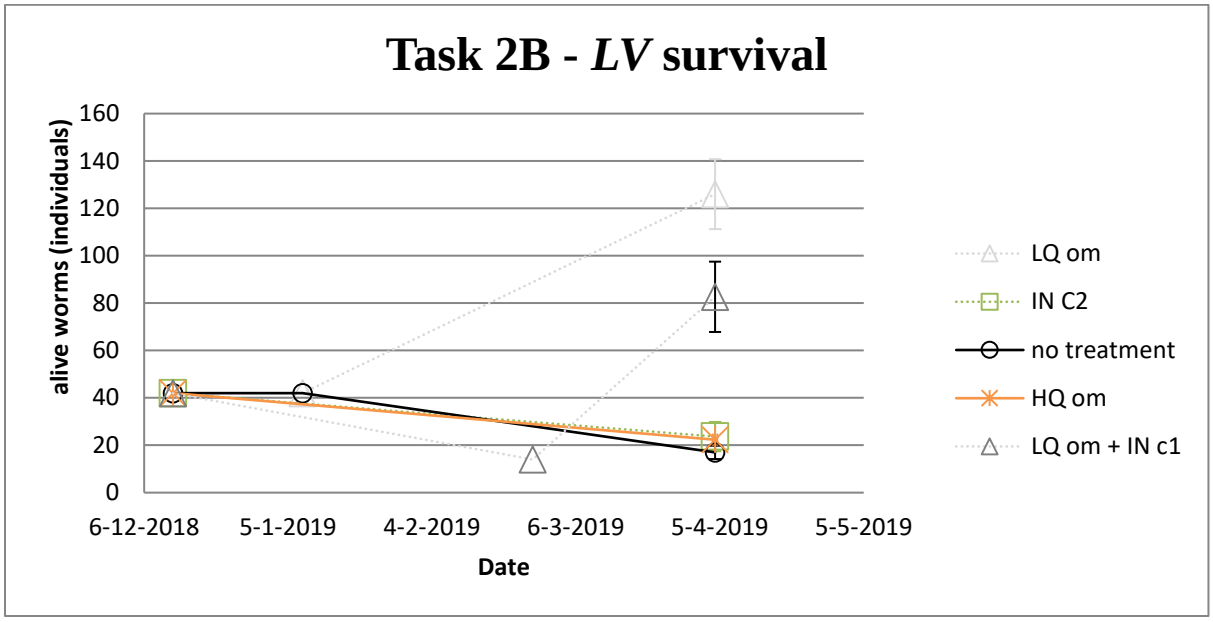

Figure 4.17. Alive *LV* worms as a function of time found for the additives tested in FFT. The abbreviations in the legend are introduced in the methodology section, in particular in section 3.3.2. The error bars account for the variability between triplicates.

4.2.3 Task 2C: non-saturated conditions

Changes in worms population from a initial 39 individuals were measured after 3 weeks in both saturated and unsaturated conditions for FFT. In unsaturated conditions 200 *LV* and 2500 *Tubifex* worm individuals were found alive. In saturated conditions 300 *LV*, and 250 *Tubifex*. This contradicts everything else we found so far reproduction wise, as in all our other tests we always measured a decline in 20% of the population after 3 weeks. Here we see a increase of factor 5 in unsaturated conditions and factor 8 in saturated for *LV*, with a factor 50 and a factor 6 respectively for *Tubifex*. Let us disregard the factor 50 obtained for *Tubifex*, as it is not the focus of this study anymore. As for the increase of a factor 5 to 8 exhibited by *LV*, we hypothesize that aging of the tailings (see section 3.3.3: FFT used in these test was these from test FFT1 at section 4.1.2, which dewatered and consolidated in the absence of additives or treatments over 5 months with water on top) may have triggered the development of a different bacterial community that is more favorable to the worms. This is of course just a hypothesis as we have no further data to sustain it, but it opens the door for considering other factors to influence the reproduction of worms beyond these considered in this study. Moreover, results were similar (factor 5 reproduction vs almost factor 8) for unsaturated and saturated conditions for *LV*, indicating that the technology could potentially work in non saturated environments as well (e.g. drying or ripening fields, but also a non-saturated region of a tailings pond).

4.3 Task 3: small-scale column testing with for environmental parameters with additives

The incorporation of straw in the small-scale columns resulted problematic initially. For reasons related with upscaling (from beaker scale) and tailings-straw homogeneity, experiments had to be repeated numerous times (in fact from Jan-2019 till Jun-2020) until the recipe worked as envisioned with respect to dewatering (in short, too much straw can lead to methanogenesis and swelling; we had to learn to design and handle larger volumes of straw + tailings mixtures). As a result of this learning process, the amount of straw for the small-scale columns was changed from 3% (as used in Task2) to 0.5%. Let's now disregard all tests from the learning process in

this section of the report, and focus on the experiments introduced in Table 4.9. These serve to the project objectives (successful dewatering and strengthening of tailings), and were obtained upon mastering the handling and the design of straw (0.5%) in a successful manner towards dewatering. Nevertheless, most of the tailings were consumed in Task 1 to 3 and throughout the process of scaling up for straw mixtures, leaving out of our test matrix a) a experiment with only TT and straw; and b) a experiment with FFT, straw and *Tubifex*.

Test id	**FFT 10 + straw**	**FFT 11 + straw**	**TT 14 + straw**	**TT 15 + straw**
Temperature	20°C	20°C	20°C	20°C
Initial concentration (g/l)	348	348	580.5	580.5
Initial sediment thickness (cm)	30	30	30	30
Initial SC (%)	29	29	43	43
worms	no	*LV*	*Tubifex*	*LV*

Table 4.9. Overview of small-scale columns in Task 3, where the effect of the survival strategies from Task 2 (addition of straw) is incorporated.

4.3.1 Solids content evolution

Figure 4.18 provides a comparison between the S_c evolution of column FFT 11, where 0.5% (in dry mass) of straw and 39 *LV* individuals were added (equivalent to 5000 individuals/m^2, or to 16.5 10^3 individuals/m^3 of tailings, or to 500 g of worms/m^3 of tailings), with the S_c evolution of column FFT 10, which also contained 0.5% straw but which did not have any worms in it. The initial conditions of these two columns were identical, with the exception of the presence of worms. For the reader's reference, please note that 4 10^4 minutes is approximately a month, and thus that 3 months have passed at 12 10^4 min, with a final experiment time of 3.5 months. For the studied initial conditions ($S_{c,0}$=29%, h_0=30cm), the increase in S_c after 3.5 months is a factor 1.6 larger for the *LV* treated tailings (from 29% to 34% for *LV*, thus a increase in S_c of 5%; from 29% to 32% for self-weight consolidation only, thus a increase in S_c of 3%). The increase in S_c is close (but smaller) than this as obtained from Figure 4.5 (where *LV* with and without worms is studied in FFT). The final S_c for both worms and no worms is, for FFT, 1 to 2% smaller than in the absence of straw. As in the experiments with no straw, the largest difference between the dewatering rates with and without worms occurs over the first 0.5 months (2 10^4). The survival of worms after 3.5 was only of 1 individual. Survival results cannot be directly compared with these in Task 2 as we changed the amount of straw from 3% (Task 2) to 0.5% (Task 3). In any case, the results of the FFT tests contradict the general statement of worms surviving and reproducing under straw. Despite the (in principle) underperformance when compared with absence of straw, the treatment still offers advantages to the absence of straw, as it will be presented in the next section.

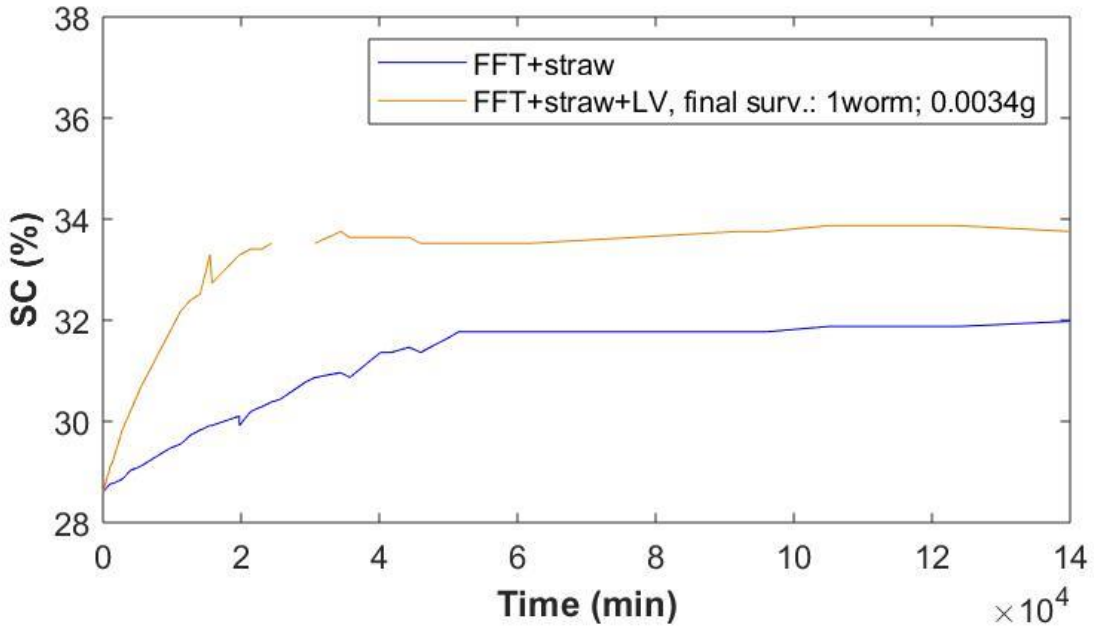

Figure 4.18. S_c evolution as a function of time for FFT, LV worms, and addition of straw. The results constitute a comparison between column FFT 10 (with straw but no worms), and column FFT 11 (with straw and *LV* worms). 4 x 10^4 minutes is approximately a month, and thus the total experiment time is 3.5 months. (this figure is to be compared with the lower panel of Figure 4.2: worms in FFT).

Figure 4.19 provides a comparison between the S_c evolution of column TT 14, where 0.5% (in dry mass) of straw and 39 *Tubifex* individuals were added (equivalent to 5000 individuals/m^2, or to 16.5 10^3 individuals/m^3 of tailings, or to 500 g of worms/m^3 of tailings), with the S_c evolution of column TT 15, which also contained 0.5% straw but which had 39 *LV* individuals. The initial conditions of these two columns were identical, with the exception of the type of worms present in the tailings. For the reader's reference, please note that 4 10^4 minutes is approximately a month, and thus that 3 months have passed at 12 10^4 min, with a final experiment time of 3.5 months. For the studied initial conditions ($S_{c,0}$=43%, h_0=30cm), S_c after 3.5 months exhibits an increase from 43% to 60% for *Tubifex* worms and straw and from 43% to 56% for *LV* and straw. These are very similar figures to these obtained from the non-straw tests in Task 1 (see Figure 4.3 and 4.6). Assuming that a hypothetical TT and straw column would have also resulted in similar results as in the absence of straw (which is the case for all other treatments), a *Tubifex* and straw treated bed would have exhibited a factor 2 larger relative increase in S_c than in the absence of worms, and a *LV* and straw treated bed would have exhibited a factor 1.6 larger relative increase in S_c than in the absence of worms. Finally, 45 *LV* individuals were found alive after the test in the straw and *LV* column, with only 5 being found alive for *Tubifex* and straw.

This means that *LV* worms reproduced in TT and straw, arriving to a final number of individuals that is larger than the initial number.

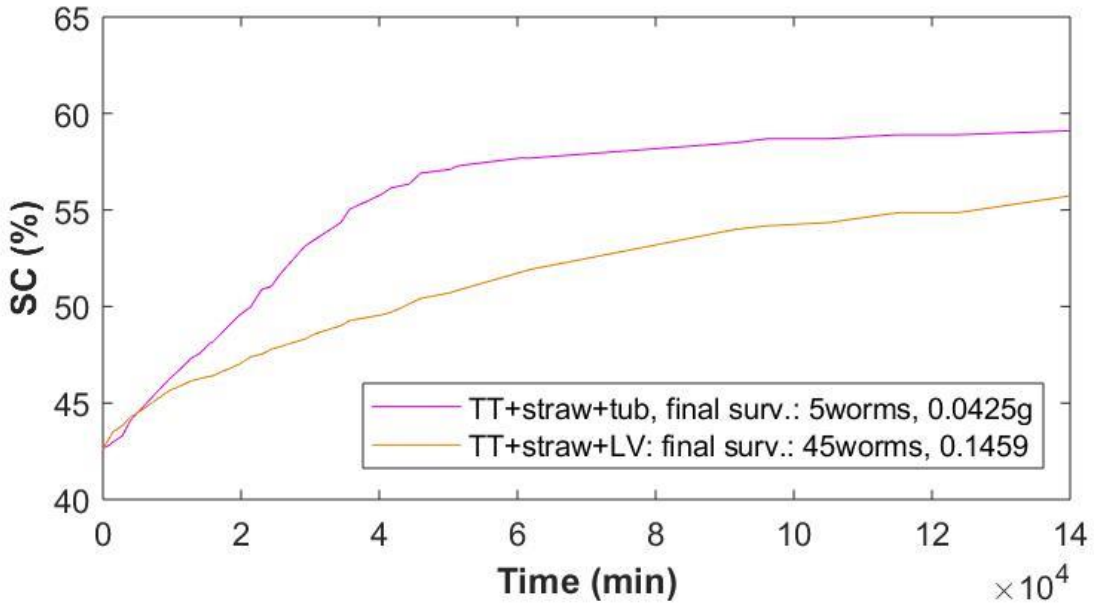

Figure 4.19. S_c evolution as a function of time for TT, LV and *Tubifex* worms, and addition of straw. The results constitute a comparison between column TT 14 (with straw and *Tubifex* worms), and column TT 15 (with straw and *LV* worms). 4 x 10^4 minutes is approximately a month, and thus the total experiment time is 3.5 months. Not enough tailings were left to perform a reference for TT and straw only.

4.3.2 Tailings properties evolution

A) Strength

Figure 4.20 shows a comparison between the undrained peak shear strength measured after 3.5 months consolidation in a column identical to FFT 10 (with straw but no worms) but with 3% straw instead of 0.5%, and a column identical to FFT 11 (with straw and *LV* worms) but with 3% straw instead of 0.5%. The column with FFT and straw displays a strength ranging from 200 Pa to 600 Pa, and increasing over depth. These figures are substantially larger than in the absence of straw, where strengths ranging from 40 Pa to 80 Pa were obtained. Introducing *LV* worms in a mixture of FFT and straw further increases the exhibited strength, reaching 450 Pa to 1500 Pa. Note that the 1500 Pa is recorded for a depth where there are no measurements with no worms. For all other depths, the strength in *LV* treated samples is larger. Also, 1500 Pa is the largest strength measured in the current project, and was achieved by only adding 0.5% of straw (in dry mass of tailings) and 0.5 g of worms (per liter of tailings). Finally, the almost 0.5 kPa (vs 0.25 kPa in the absence of worms) at the surface are particularly interesting for future application of this technology.

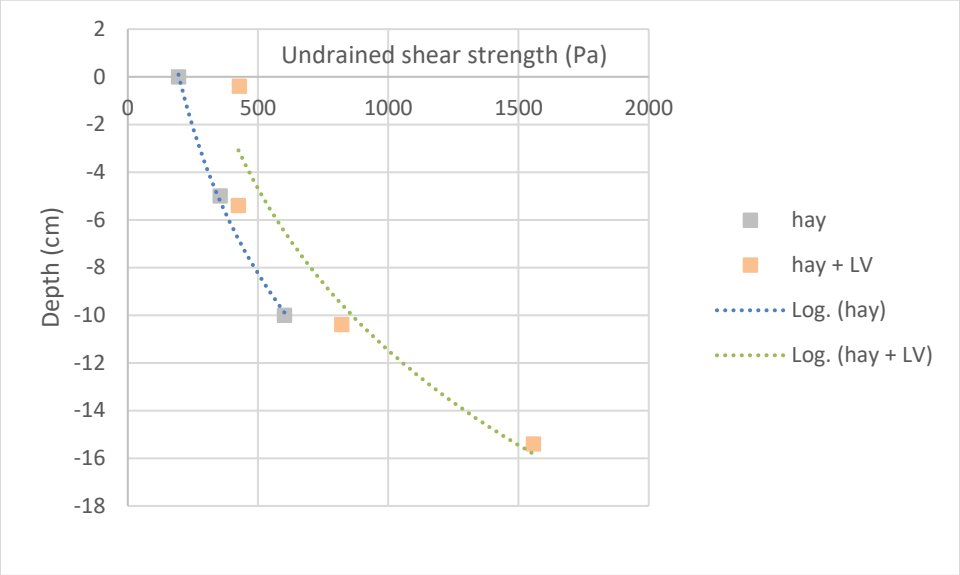

Figure 4.20. Undrained peak shear strength measured after 3.5 months consolidation in a column identical to FFT 10 (with straw but no worms) but with 3% of straw instead of 0.5%, and a column identical to FFT 11 (with straw and *LV* worms), but with 3% of straw instead of 0.5%.

4.4 Task 4: large scale column testing (at University of Alberta)

4.4.1 Preliminary tests

Preliminary tests were performed from January to September 2020. The initial tests involved familiarization with worms handling as per Deltares' advice, which included simplified repetition of Deltares' beaker testing for *LV* survival and reproduction with their recommended concentration of straw amendment (0.5 %).

Briefly, 1 L glass beakers were filled with 0.5 L of FFT and TT amended with shredded straw and covered with 0.5 L of water; 17 worms (double the number to practice worm counting) were added to the experimental beakers. The oats straw was manually cut to 3-5 mm pieces using a paper cutter. Beakers were loosely covered with foil to prevent extensive evaporation, but allow oxygen penetration from the air. Two beakers with just FFT/TT, two beakers amended with straw (0.5 % of tailings dry weight) and three beakers amended with straw and worms (14 in total) were established initially to mimic future large-scale column testing. Beakers amended with straw and worms were sacrificed to allow for worms counting after 1, 2 and 3 months of incubation. The obtained results (FFT: 7 live worms, 12 live worms, 1 dead worm; TT: 13 live worms, 1 live worm, 2 live worms and 1 dead worm counted respectively after 1, 2 and 3 months of incubation) showed very poor worm survival dynamics. Therefore additional beakers were established on June and July 2020, as per Deltares' advice at the June 2020 stewardship meeting, to investigate if different straw concentration will positively affect worm survival. For this experiment, the beakers with straw (0.2%, 1%, 2%) and worm amendments only were set up, without controls. In total, 30 additional tests were conducted with different straw concentration. These additional tests continued for 2 months and beakers with 0.2% of straw concentration showed the best performance in terms of worm survival (up to 15 live worms were counted after two months of incubation). The additional promising result of the experiments with 0.2% straw was that worms significantly smaller (baby-worms) than were used initially were detected after

two months of incubation. This was evidence that the worms were not only dying, but also reproducing, however little. As such, these conditions were used to set-up the large-scale column tests described in the next section.

4.4.2 Results from large scale column tests

The initial experiment design (two treatments in duplicate[1]) was improved: three controls were added to distinguish the worms' effect from gravitational settling (FFT/TT control) and microbially accelerated settling (FFT/TT + 0.2% straw control), as well as positive control (FFT/TT + worms). See Figure 4.21 for an overview of treatments and control.

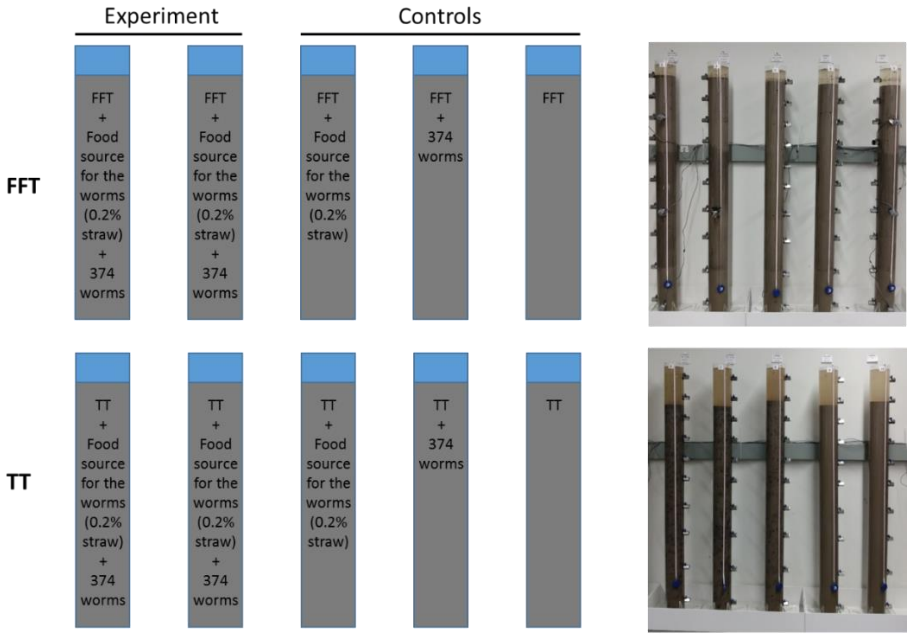

Figure 4.21. Experiment layout shows 10 columns: 5 with FFT and 5 with TT capped with artificial OSPW water. Left panels – schematic column drawing; right panels – photographs of the columns during incubation in the same order as schematic drawings.

A) Consolidation (mudline) monitoring

The experimental columns, treated with straw and worms, demonstrated better consolidation for both tailings (black and red lines on Figure 4.22) as compared to the controls. For FFT consolidation reached up to 24% and for TT the consolidation was better – up to 29%. All experimental columns outperformed the control columns. At the time of the end of the experiments, all columns were still undergoing consolidation.

[1] Planned as such in the proposal, not in the experimental description at the introduction of this report where it have been modified accordingly

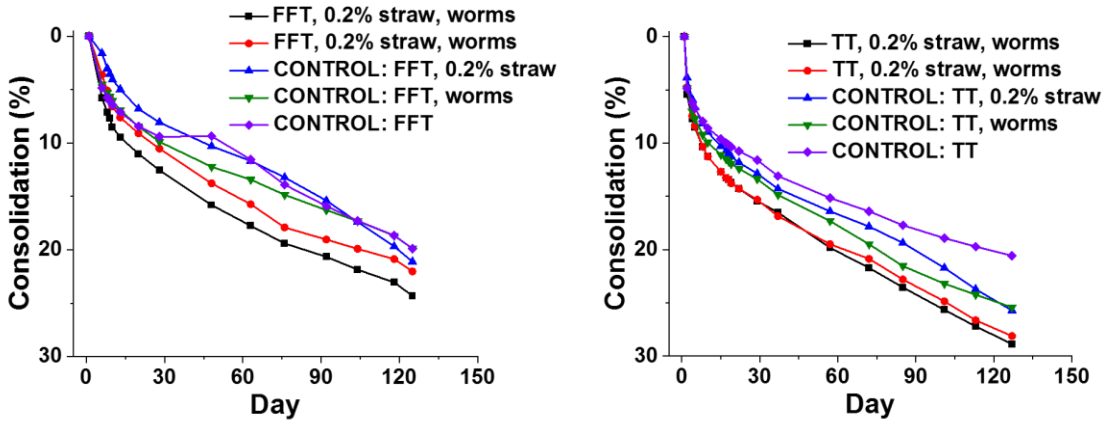

Figure 4.22. Consolidation of solids layer measured below the mud line in the columns. Values were calculated as a percentage of the initial volume of tailings placed in the columns.

Note that the results in Figure 4.22 are not directly comparable with any of the results throughout Tasks 1 to 3 (not only because the tailings are different, but also because the Task 1 to 3 results are not plotted in % of consolidation), with the exception of (indirectly) the settling interface results in Figures 4.2 and 4.3. Nevertheless, trends can be compared. In general, Task 1 to 3 showed that worm treated samples and worm and straw treated samples achieved an equivalent amount of consolidation, only faster in the presence of straw. In the larger columns, further compaction is systematically achieved in the presence of straw and worms, followed by worms only with the tailings only and the tailings and straw columns showing the smallest degree of compaction throughout all columns. It seems therefore that the increase in depth and scale helped the straw and worms treatment to further improve dewatering.

B) Solids content

Solids content increased in all columns during the experiment compared to day 0 due to gravitational settling. Increases of 28 to 40% and 40 to 56% in FFT and TT, respectively. Results cannot be directly compared with these from Tasks 1 to 3 in this report, as these were obtained for a new and different batch of sediments. Higher solid content was detected in top layers of experimental columns (where worms were most likely active) compared to the samples taken from middle and bottom layer of tailings. This tendency was more pronounced in FFT as compared to TT. According to T-test, there was a significant difference between top layers in the FFT experimental columns (39.93$\pm$0.74 and 38.90$\pm$0.16) and the top layer in all the control columns (36.62$\pm$0.22, 33.94$\pm$0.01 and 36.17$\pm$0.15); p=0.05 and TT experimental columns (55.26$\pm$0.04 and 55.45$\pm$0.06) and the top layer in all the control columns (53.52$\pm$0.02, 54.71$\pm$0.03 and 51.45$\pm$0.10); p=0.05.

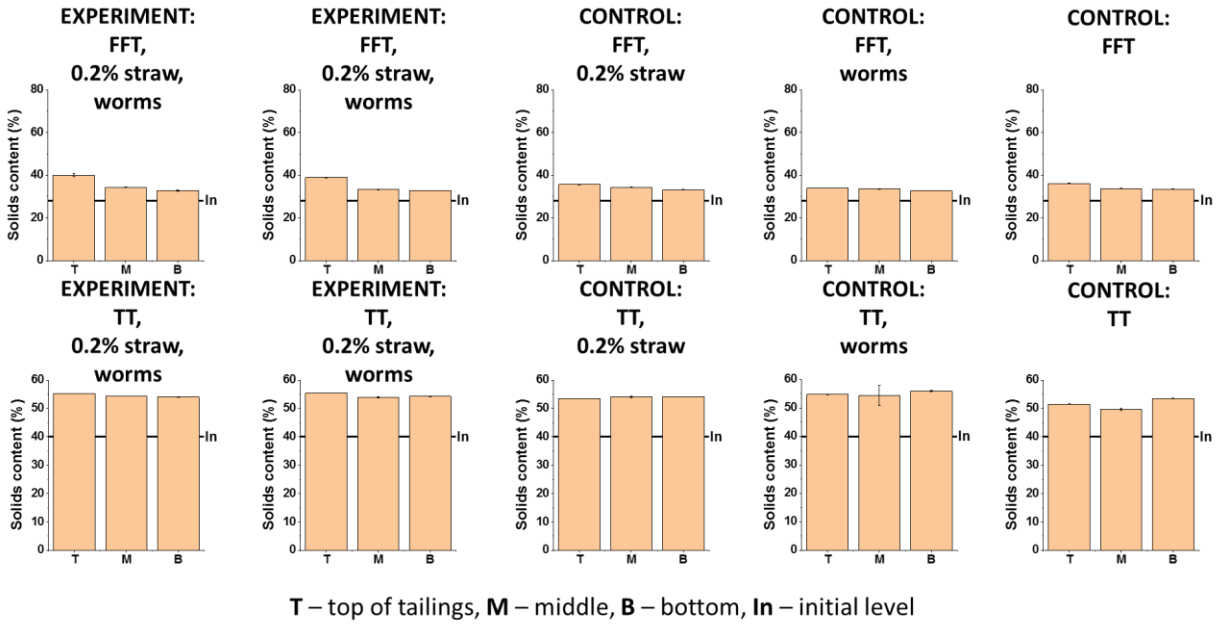

Figure 4.23. Solids content in the initial samples (represented as a horizontal solid line labelled "In") and in the samples collected at the end of the experiment for the top (T), middle (M) and bottom (B) layers of the tailings column. Data is shown as an average of two replicate samples with deviation bars representing +/- one standard deviation.

C) Peak undrained shear strength

Shear strength was measured for the initial samples (horizontal line on graphs, Fig. 5) and for the samples collected at the end of the experiment (vertical bars on graphs). It significantly increased for both FFT and TT from 7.92 Pa and 7.56 Pa to 65 Pa and 159 Pa, respectively. Columns amended with straw and worms show the best performance compared to the controls and TT did better than FFT showing higher shear strength at the end of the experiment.

According to T-test, there was a significant difference between top layers in the FFT experimental columns (65.47$\pm$6.88 and 58.00$\pm$3.97) and the top layer in all the control columns (38.90$\pm$0.85, 12.33$\pm$0.65 and 10.63$\pm$0.53); p=0.05 and TT experimental columns (157.33$\pm$8.13 and 158.53$\pm$5.28) and the top layer in all the control columns (81.17$\pm$1.60, 39.70$\pm$0.64 and 30.80$\pm$1.20); p=0.05. T-tests were not performed for middle and bottom data, but the data does exhibit the same trends and relative difference as in the case of the studied top layer. In general, results indicate a conceptual picture that is consistent with the results of Task 1 to 3: worms and straw, together, increase the peak undrained shear strength relative to the straw treatment only, which otherwise results in larger peak undrained shear strengths than any of the controls. The maximum peak undrained shear strength measured in this Task is one order of magnitude smaller than these of Task 3. This difference can be explained by: a) the tailings are different; b) the large scale columns are still undergoing consolidation at the time of the strength measurements, whereas the small scale columns were already in equilibrium; c) a smaller dosage of straw in Task 3 (0.2% in Task 4, 3% in Task 3).

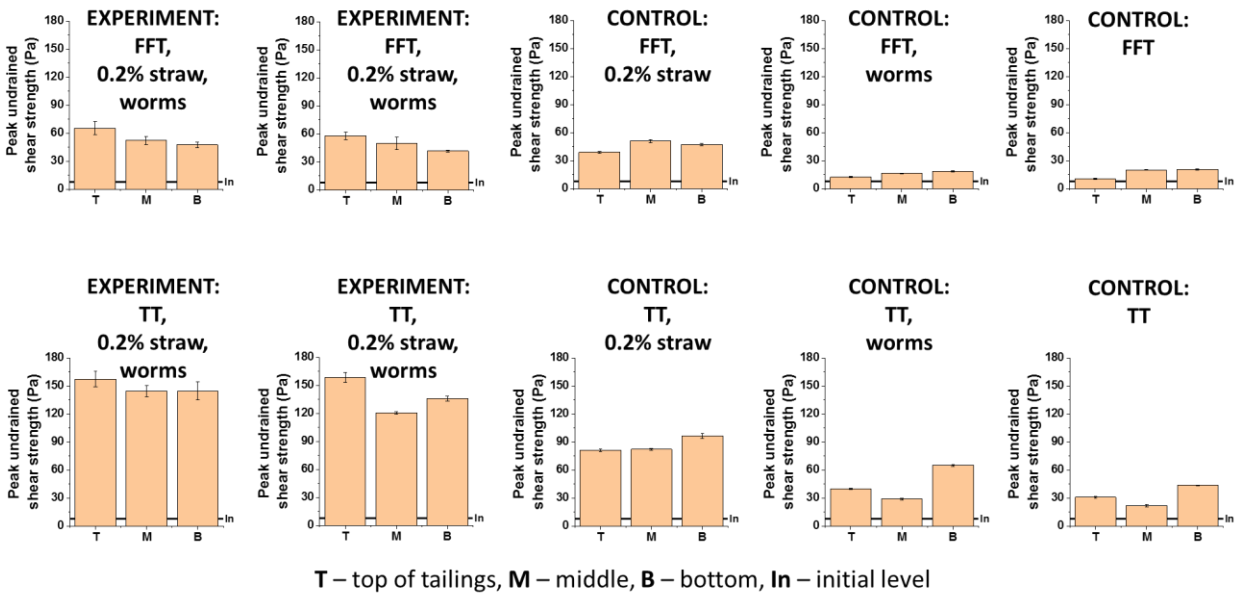

Figure 4.24. Peak undrained shear strength in the initial samples (represented as a horizontal solid line) and in the samples collected at the end of the experiment for the top (T), middle (M) and bottom (B) layers of the tailings columns. Data is shown as an average of three replicate samples with deviation bars representing +/- one standard deviation.

D) Pore water pressure

During the whole course of the experiment the pore water pressure readings were highest at the bottom of the columns (~ at level 19 kPa for FFT amended with straw and worms), and lowest at the top (~ at level 4 kPa for the same column) (Figure 4.25). As tailings consolidate, the pore water pressures decreased. So over the course of the experiment, the pore water pressure readings all decreased. This is evidence of the tailings consolidating/dewatering (water is moving upward, out of the tailings, so the water pressure between the solid particles is decreasing). The presence of straw interfered with the power water pressure readings to a certain extent.

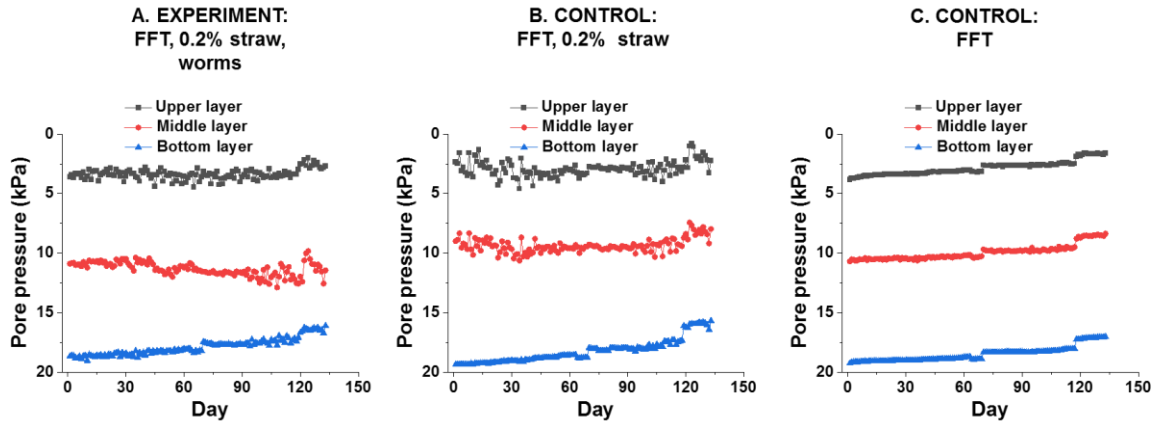

Figure 4.25. Pore pressure monitoring in the upper, middle and bottom layers during the course of the experiment through the ports equipped with pressure transducers

E) pH monitoring

The pH in cap water was ~one unit higher than the pH in tailings during the whole course of the experiment (Figure 4.26). The pH level in all columns with straw decreased fast. This phenomenon was described before as an effect of active methanogenesis. pH decline started very quickly because we used straw pre-soaked in water (activated) and once methanogenesis started, pH dropped below 7. This is typical behavior for the tailings with active methanogenesis (Siddique et al. 2014a) and we saw it before in all our experiments with microbial activity stimulation. In all control columns without straw, the pH level did not change during the incubation. Cap water pH followed the tailings trend.

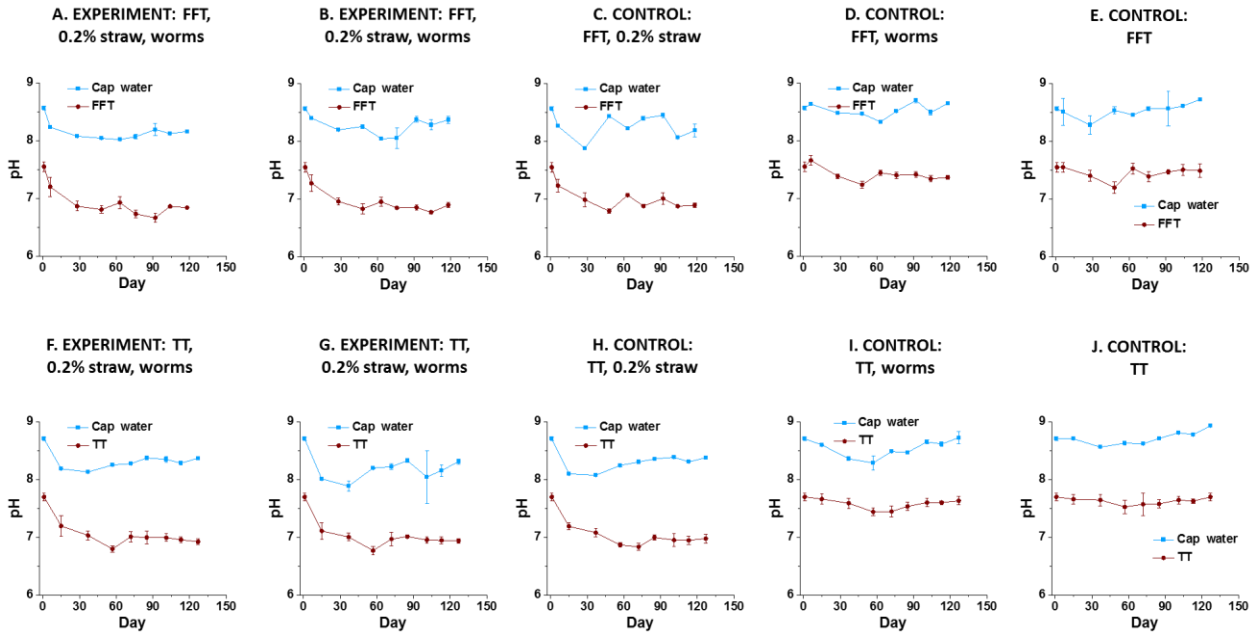

Figure 4.26. pH of cap water (blue lines) and bulk tailings (brown line), measured *in situ* at ports of all columns during incubation. Values represent the average from of measurements taken at each port *in situ* with pH probe with deviation bars representing +/- one standard deviation.

H) ORP monitoring

ORP (oxidation / reduction potential) was used to identify anaerobiosis at or near the water column and sediment interface. Numerically positive ORP represents an oxic environment, negative ORP characterize anoxic conditions; however in most environmental media, redox reactions are not in equilibrium due to multiple redox species. Consequently, redox measurements can generally be considered semi quantitative in environmental media (USEPA, 2017).

Our ORP monitoring data shows cap water in all column was suboxic at the beginning and the end of the experiment (+100 mV). During the active methanogenesis, cap water became anoxic (-100-180 mV) both FFT and TT tailings, amended with straw or unamended are strictly anaerobic (-300mV), which makes them not a perfect substrate for *LV* worms living. Straw addition makes tailings environment even more anoxic, -300mV vs -200mV (Figure 4.27, panels A, B, C vs D and E; F, G, H vs J and K). With aerobic environment in the cap water worms can possibly enrich the upper layer of the tailings with the oxygen through wormholes, making environment more suitable for living. Finally, error bars in the ORP data are in general only

visible in the controls with only worms (as well as in 3 points in time in the control with only TT). A possible explanation to this observation could be that worms trigger redox gradients over depth (the displayed measurement is in fact the average of measurements at all ports). We lack of an explanation for the reason why this also happen at times in the control with only TT.

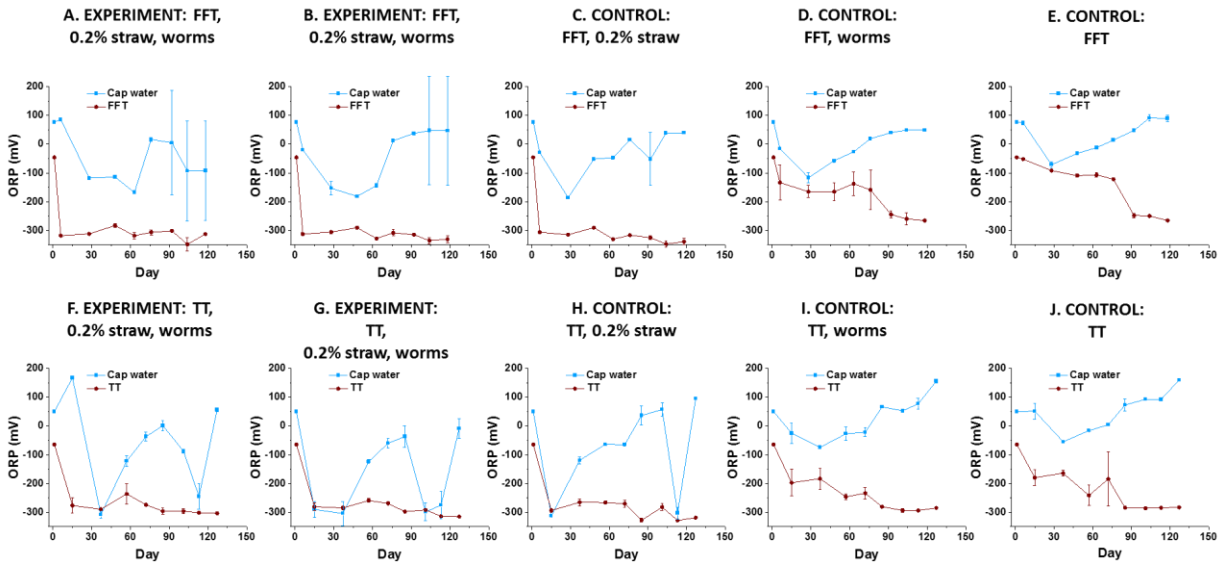

Figure 4.27. Redox potential (ORP) of cap water (blue lines) and bulk tailings (brown line), measured *in situ* at ports of all columns during incubation. Values represent the average from measurements taken at each port *in situ* with deviation bars representing +/- one standard deviation.

I) Alkalinity

Similar to pH, alkalinity in columns amended with straw were higher compared to control columns. For FFT the alkalinity reached 650-700 mg/L $CaCO_3$, for TT it was 700-800 mg/L $CaCO_3$. There were no significant differences between the tailing layers and cap water. Increase in alkalinity is also coupled with active methanogenesis followed by pH decreasing and dissolving the carbonate minerals. These mechanisms were discussed in detail in Siddique et al., 2014a and Siddique et al., 2014b.

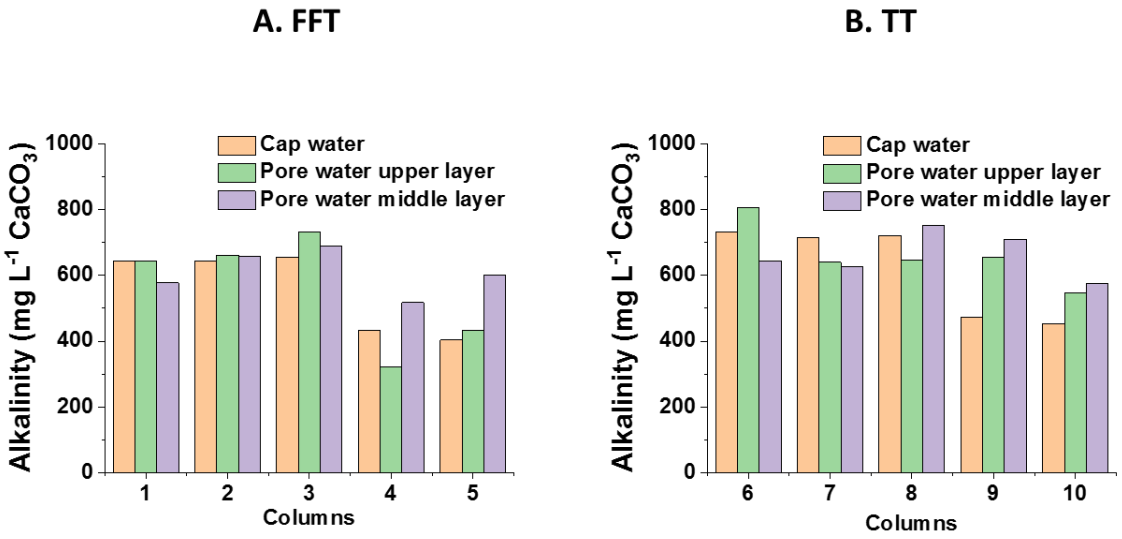

Figure 4.28. Alkalinity in cap water and pore water from upper and middle tailings layers of all columns: left panel – FFT, columns 1-5, right panel - TT columns 6-10 at the end of the experiment. 1, 2 and 6, 7 – experimental columns amended with 0.2 % straw and worms; 3 and 8 – control columns amended with 0.2 % straw; 4 and 9 – control columns amended with worms only; 5 and 10 – control columns with FFT and TT only, respectively.

J) Dissolved organic compounds

Dissolved organic compounds (DOC) content was similar in cap water of all columns – in FFT columns DOC was ~4 mg/L, in TT columns it was slightly higher at ~4.5 mg/L. In pore water of control columns for both FFT and TT, DOC content was similar to cap water. In columns where straw was added DOC increase to 5 mg/l for FFT and 6mg/L for TT. It is explainable because straw contains high amount of organic carbon that can be dissolved and enrich pore water with DOC. In the TT experiments, worms and straw resulted in more DOC in the pore water than only straw.

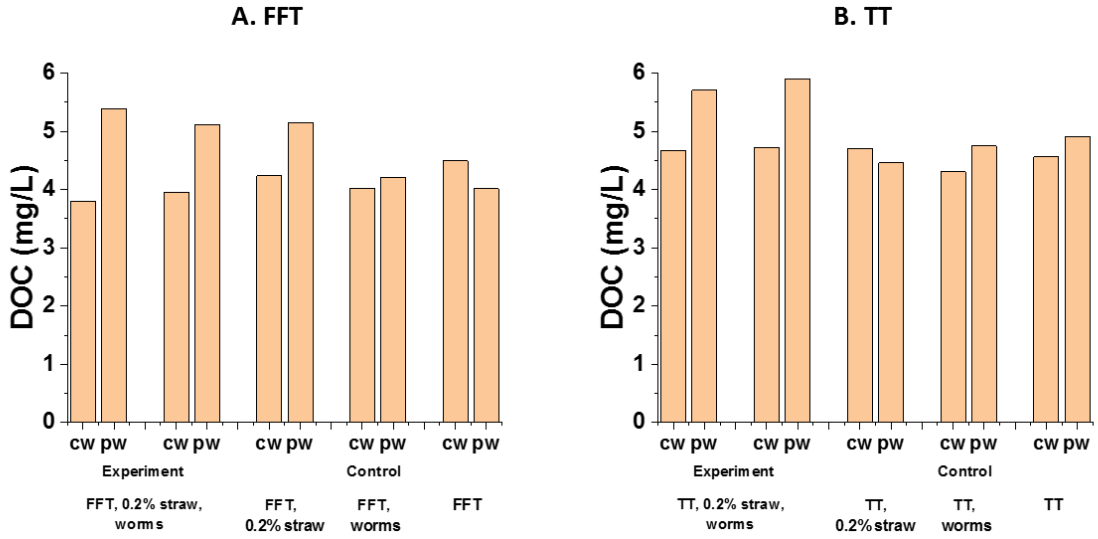

Figure 4.29. Dissolved organic compounds in cap water (cw) and pore water (pw) of samples collected from the columns at the end of the experiment. Left panel – FFT columns; right panel – TT columns.

K) Chemical oxygen demand

The general trend for TT columns was the higher COD for pore water compare to cap water. There was no correlation with straw/worm amendment or methanogenesis because highest COD was detected in control columns (TT alone, at 320 mg/L) and in experimental TT columns COD varied from 200 to 310 mg/L. In FFT columns, highest COD was determined in control columns, FFT alone and with worms, 300 mg/L and 420 mg/L, respectively. In experimental FFT columns it varied (200-320 mg/L) so COD was not related with the experimental treatments.

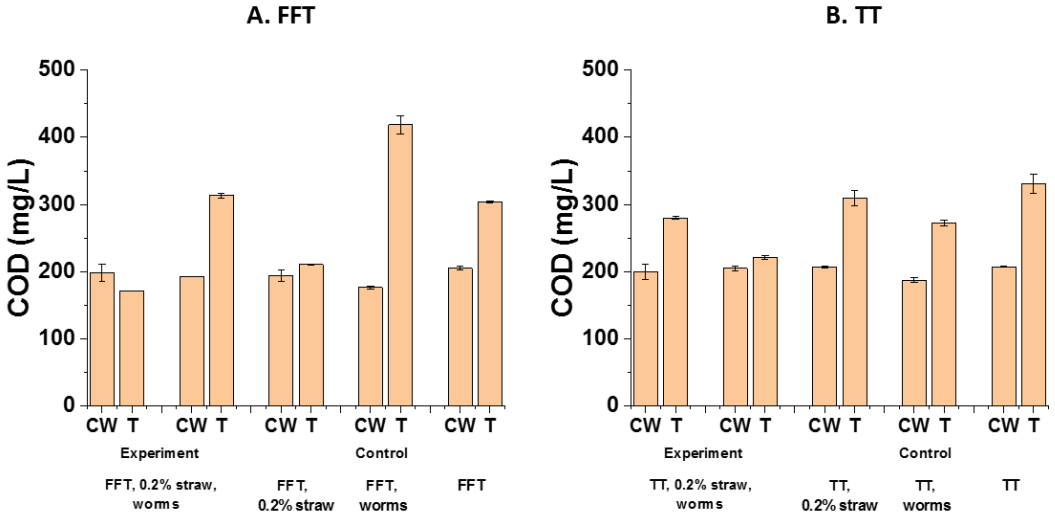

Figure 4.30**.** Chemical oxygen demand in cap water (CW) and bulk tailings of samples (T) collected from the columns at the end of the experiment. Left panel – FFT columns; right panel – TT columns. Data is shown as an average of two replicate samples with deviation bars representing +/- one standard deviation.

L) Microbial community structure

We were not able to measure the methane production in the columns because they were open at the top to let the worms breathe. However, the straw amended column produced bubbles that were visually detected in the tailings layer (Figure 4.31) and then active methanogenic processes were confirmed by analyzing the microbial community for the initial samples and at the end of the experiment. These methanogenic bubbles did not seem to affect consolidation in the tests. At larger time scales it remains unknown if bubbles production would continue and if they would affect consolidation. Two groups of microbes were detected, Archea and Bacteria. More than 500 taxonomic units were found, but in Table 4.10 we show only groups of microbes with an abundance of more than 3% of total readings.

Figure 4.31. Photographs of amended column during incubation: Pores in tailings created by biogases – methane and carbon dioxide- during active methanogenesis

The shift in microbial community structure in straw-amended columns was detected at the end of the experiment by the sequencing of partial 16S rRNA genes (Table 4.10). Two orders of methanogenic Archaea increased in numbers, hydrogenotrophic *Methanomicrobiales* and acetoclastic *Methanosarcinales* and became key players in microbial metabolism in these columns. Abundance of methanogens increased from 0 in initial tailings to 22-35% in FFT and 32-40% in TT. In columns without straw-amendment, the microbial community didn't change much and left similar to initial; mostly *Proteobactaeria* were detected (*Burkholderiales* and *Spirochaetales*). Only *Chloroflexi* increased in numbers in the control columns without straw that are usually abundant in tailing ponds under anaerobic conditions. Other important orders is strictly anaerobic fermentors sulfate- and/or sulfur-reducing bacteria *Syntrophobacterales* (*Syntrophus*) occupied up to 4% in FFT and 10% in TT of the bacterial community in control column (compare to 1-2% in initial tailings).

Table 4.10. Relative abundance of archea and bacterial 16S rRNA gene sequences in initial tailings and collected from upper layers of columns at the end of the experiment. Numbers represent the percentage of total bacterial reads. Only OTU with abundance >3% are presented in detail.

Taxonomy	FFT1	FFT2	FFT3	FFT4	FFT5	TT6	TT7	TT8	TT9	TT10	FFT initial	TT initial
Archaea;Halobacterota;Methanomicrobia	6.57	7.56	7.43	0.00	0.00	4.89	3.14	3.08	0.00	0.00	0.00	0.00
Archaea;Halobacterota;Methanosarcinia;Methanosarciniales;Methanosarcinaceae;Methanosarcina	25.45	34.65	22.09	0.00	0.01	39.60	32.36	32.82	0.00	0.00	0.00	0.00
Bacteria;Bacteroidota;Bacteroidia;Bacteroidales	2.39	7.81	4.39	0.17	0.20	6.83	11.93	15.38	0.20	0.43	0.83	0.80
Bacteria;Chloroflexi	1.56	1.11	2.37	12.11	11.94	1.62	1.81	1.95	10.53	12.41	6.93	6.25
Bacteria;Deferrisomatota;Defferrisomatia;Defferrisomatales;Defferrisomataceae;Deferrisoma	0.06	0.03	0.08	0.40	0.36	0.08	0.09	0.13	3.09	3.26	0.11	0.14
Bacteria;Desulfobacterota;Desulfuromonadia	2.13	4.91	7.19	2.24	0.29	2.45	2.65	2.72	0.38	0.24	0.22	0.10
Bacteria;Desulfobacterota;Syntrophia;Syntrophales;Syntrophaceae;Syntrophus	0.10	0.23	0.45	4.07	2.69	0.13	0.14	0.15	6.03	10.05	1.43	2.86
Bacteria;Firmicutes	3.67	2.95	1.89	0.51	0.00	0.26	0.20	0.08	3.70	0.02	0.00	0.00
Bacteria;Nitrospirota;Thermodesulfovibrionia	0.15	0.18	0.29	3.90	1.97	0.15	0.19	0.15	2.58	1.32	0.65	1.26
Bacteria;Proteobacteria;Alphaproteobacteria	0.22	0.41	0.64	2.96	3.42	0.33	0.44	0.45	2.55	3.06	2.25	1.73
Bacteria;Proteobacteria;Gammaproteobacteria;Burkholderiales	17.39	4.14	6.80	18.75	20.54	2.54	2.74	2.93	15.19	13.35	31.77	32.54
Bacteria;Proteobacteria;Gammaproteobacteria	2.13	3.15	5.68	19.92	26.16	2.52	2.19	2.32	11.84	14.70	22.12	17.99
Bacteria;Spirochaetota;Spirochaetia;Spirochaetales;Spirochaetaceae;Treponema	0.44	2.07	0.98	0.01	0.00	3.40	8.12	9.09	0.02	0.01	0.01	0.00
Other <3%	37.74	30.78	39.72	34.96	32.40	35.18	34.00	28.77	43.88	41.15	33.66	36.32

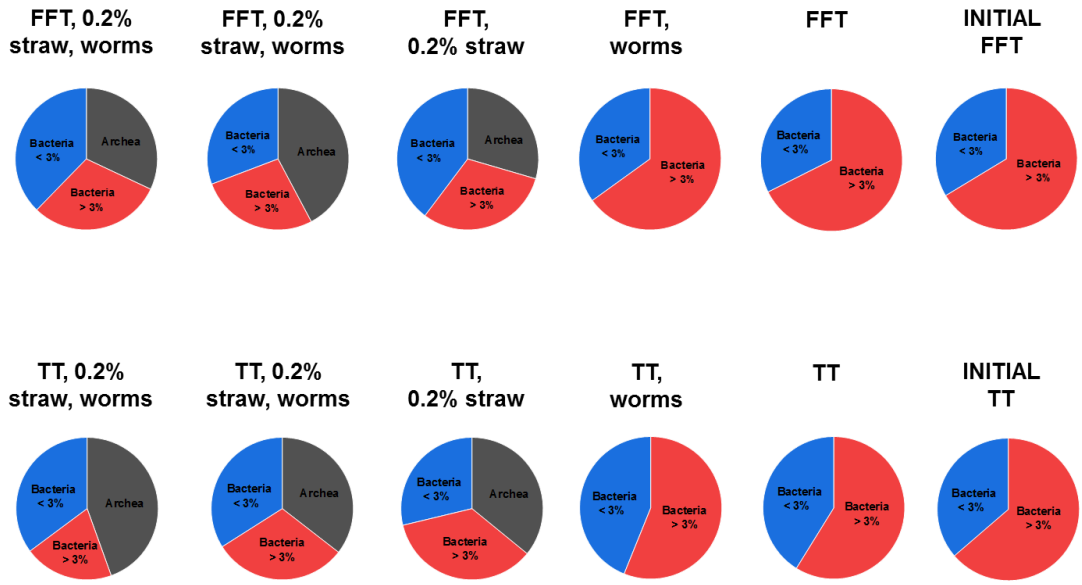

Figure 4.32. Increasing of Archaea community compare to Bacteria community in straw-amended tailings after incubation in columns. The grey are represents Archea (15 OTU) and blue - Bacteria < 3%(520 OTU) and red - Bacteria > 3 % (30 OTU).

M) LV worms survival

Worms's survival was the most important parameter due to their potential consolidation activity. We counted the worms at the end of the experiment; the only column where we found the worms was TT control column without added straw. Less than 10% of 374 initially added worms

survived. The explanation of low worm survival might be: (i) the lack of oxygen in the columns due to the oxygen demand and the active methane production; (ii) toxic environment of the tailings.

Another observation was the limited depth of tailing that worms seem to have reached, or in other words where tunnels were visible with the naked eye; most of the visible tunnels were concentrated in the first 10-15 cm of tailings (Figure 4.33). Nevertheless, the difference in consolidation registered for the TT columns between treatments (straw and worms) and the control with only FFT was of 10% of the initial tailings thickness, which equals 18.5 cm. Worms are therefore capable to result in a net compaction larger than their apparent penetration depth.

Figure 4.33. Photographs of worm-amended column during incubation: black spots in tailings are areas of accumulation of dead worms.

Table 4.11. Worm count in experimental columns at the end of the experiment. Initially 374 worms were introduced to the columns.

EXPERIMENT: FFT, 0.2% straw, worms	EXPERIMENT: FFT, 0.2% straw, worms	CONTROL: FFT, 0.2% straw	CONTROL: FFT, worms	CONTROL: FFT
0	0	-	0	-

EXPERIMENT: TT, 0.2% straw, worms	EXPERIMENT: TT, 0.2% straw, worms	CONTROL: TT, 0.2% straw	CONTROL: TT, worms	CONTROL: TT
0	0	-	23	-

N) Microtox acute toxicity tests

Microtox acute toxicity analysis showed that initial tailing samples were not toxic, at the level 0.34 and 0.47TU for FFT and TT, respectively (Table 4.12). TU<1.0 indicates complete detoxification (Scott et al., 2008). Toxicity of the upper layer of tailings from each column increased significantly after adding the straw, except column with TT amended with straw and worms. We hypothesize that the fact the toxicity does not develop in the column with TT amended with straw and worms can be related to the presence of oxygen throughout the tunnel matrix made by the worms. Columns with FFT became highly toxic. Finally, adding only worms to the tailings did not result in any increase of toxicity in the columns.

Table 4.12. Microtox acute toxicity test

Sample ID	Toxicity unit (TU)	
	FFT	TT
Initial sample	0.34	0.47
Tailings + 0.2% straw + worms	high level[a]	43.48
Tailings + 0.2% straw + worms	high level	non toxic[b]
Tailings + 0.2% straw	714.29	2.09
Tailings + worms	non-toxic	non-toxic
Tailings	non-toxic	non-toxic

a Non-toxic means TU is below instrument detection limit.
b High level is above instrument detection limit.

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The results of the small-scale tests performed to fresh samples of fluid fine taling (FFT) and thickened tailing (TT) with typical in-situ densities, where only worms were added to the 30 cm thick layers of tailings, demonstrated the potential to effectively dewater and strengthen tailings of *Oligochaete* worms under a relevant range of operational parameters. The increase in Solids content (Sc) observed for *Tubifex* was up to a factor 2 larger relative to the controls in both FFT and TT (e.g. in the control a increase in Sc of 8% is observed due to self-weight consolidation, with 16% being observed with worms). *LV* resulted in a factor 1.33 in TT and 1.75 in FFT. Therefore, the performance of LV seems to vary as a function of the tailings type, which was not the case for the studied set of tailings and *Tubifex*. Finally, decreasing the ambient temperature to less than half (from 22 °C to 10 °C) did not affect the positive effect with respect to the increase in Sc.

Worms treated tailings also exhibited a larger undrained shear strength, a factor 1.5 to 3 stronger than the control without worms. The largest differences with the controls in strength were found at the tailings surface, whereas the largest strengths in absolute values were still found in depth. The strength profiles obtained under worms treatment suggest strengthening due to factors other than dewatering. These are maybe related to the oxidation of the tunnel network created by the worms. Finally, the permeability of worm treated beds was found to be 2 orders of magnitude larger than these under the exact same void ratio in the absence of worms for a range of void ratios exhibited during our actual permeability experiments (around a Sc of 35%), but ultimately conveyed to the same order of magnitude (but still larger for worms) towards the smallest void ratios. The permeability measurements also confirmed that the same permeability is exhibited in a bed with 5% more Sc than in the absence of worms. In other words, worms manage to increase the permeability of compacts beds to levels that are otherwise typical of less consolidated and more permeable beds.

All the results discussed so far were collected in experiments where only worms were added. With no other amendments, we know that the worms population slowly decays as function of time, reaching 20 to 40% of the initial population after 3 months. In this paragraph, we present the conclusions from a beaker study dedicated to improve survivability and reproduction of worms, and where a number of strategies to feed the worms and improve their reproduction were tested. We studied the effect of adding high-quality and low-quality organic matter, and of several concentrations of inorganic nutrients in the survival and reproduction of worms in 500 ml beakers of tailings. The results from all our survival tests brought two important conclusions. The first one is that after 4 months the absence of an amendment resulted in a decrease of worms population around 50%, hence better but close to the numbers obtained in previous studies. The second one is that the addition of low-quality organic matter, particularly straw pieces of 3 cm in our tests, leads to a factor 3 increase in worms population after 4 months. Note that we foresee applications of the technology where worms reproduction and-or long term survival is not necessarily needed (e.g. mine closure combined with plants), but it always remains useful to be able to know and to control the amount of worms alive in the system. The survivability tests were executed for FFT only, and were independent of the type of worm used. The fact that *LV* and *Tubifex* showed similar reproduction and survival with and without additives in FFT partially justifies the choice of *LV* as new worms for this study after the scope modification.

Later we repeated the small-scale tests, but this time by adding the amendment that results in worms reproduction. Adjustments of the amount of straw added in the columns had to be made from what obtained in the beakers (0.5 l) before arriving to a recipe that worked in the small-scale columns (2.5 l approx.). At the end, it was found that 0.5% is a more appropriate straw amount to be implemented in larger volumes of tailings, and to avoid potential methanogenesis issues (which would ultimately kill the worms). The iterations needed to adjust the amount of straw resulted in less experiments and controls in this last batch of tests (the last performed in The Netherlands), as tailings were consumed in the intermediate experiments that we used for learning. In a 30 cm thick layer of TT the performance of *Tubifex* and straw was almost identical as in this of *Tubifex* in absence of straw: Sc increases from the initial 43% to a final 60% at equilibrium, and at approximately the same pace as it did without straw too. *LV* and straw also resulted in a very similar (only marginally better) performance in TT as it did when only *LV* was studied. As for the survivability, only 12% of *Tubifex* survived, whereas 115% of *LV* was measured at the end of the tests. This means that the amendment to improve worms reproduction as obtained from the beaker tests worked in triggering worms reproduction and effectively increasing worms population, but at a smaller reproduction rate than these found in the beakers, and only for *LV*. The latter is understandable, as 1) the survivability strategy was studied for tailings 2 years younger than these re-tested for the small-scale experiments, and 2) the amount of straw was decreased to a smaller amount, justifying a smaller performance too. For a 30 cm thick layer of FFT, we only studied the effect of *LV*, which was found to be similar to what was found for *LV* and FFT only (with no straw). Nevertheless, only 1 *LV* worm was found alive in this column. For FFT we also studied a control with only FFT and straw, which confirmed that the addition of straw at the dosage discussed here does not change dewatering and consolidation behavior of tailings. Finally, the strength was measured in FFT columns treated with straw (but this time 3%), and with and without *LV*. As expected, the addition of straw does improve the undrained shear strength from what observed in the absence of straw, this is from typically 80 to 120 Pa over the upper 10 cm (after self-weight consolidation with no worms) to 200 to 500 Pa over the same depth (after self-weight consolidation in a sample containing straw). Nevertheless, *LV* further increases the measured strengths from 500 to 800 Pa.

The most successful recipe to obtain both optimal dewatering performance and worms survival in the long term was then shared with the UofA team (this is 0.5% of straw and worms), who had to study it in a larger scale (1.85 m tall columns), but in a new batch of tailings. With different tailings and (likely) different worms (though ordered as the same species and sub-subspecies, size differences were observed in specimens between North America and Europe), the UofA team had to do a preliminary study to test worms reproduction in their tailings. The latter did not result in the reproduction numbers (up to a factor 3) as obtained by the Deltares team, but only to survival over 2 months and the observation of even smaller worms that were presumed to be newborn individuals. This is to be considered when evaluating the results from the large-scale tests.

In the larger scale, worm (*LV* was the only species studied at UofA) and straw treatments resulted in the best consolidation results, in fact better than for worms only (and any other control), which constituted a difference with the findings of the small-scale tests in Deltares. In the small scale, worm treated samples exhibited increases in consolidation of 10 % in FFT (e.g. it consolidated 17% with no worms, and 27% with worms; thus 10% more with worms) and 15% in TT, and regardless of the presence of straw. In the large-scale tests, consolidation was 5 and 10% larger for FFT and TT, but for the straw and worm treated samples. The large-scale tests

with worms and straw did not only result in the largest consolidation, but also in the highest solids content in the upper layer and highest shear strength compared to control columns. The latter was recorded for an amount of straw that turned out not to be properly optimized, as it resulted in the development of methanogenesis. Even then, the worms and straw treatments managed to deliver relevant consolidation improvements, smaller but in the same order as these obtained in the small-scale tests. It is also relevant to note that the large-scale tests were not in equilibrium when they were stopped (4 months), and therefore the maximum potential to dewater tailings with worms in large scale columns is still to be quantified, with what we have so far being a intermediate conceptual picture. Particularly for the TT tests, worm and straw treatment and control with no worms showed diverging trends at the time the tests were stopped, suggesting larger differences to be observed in equilibrium.

In principle, straw is added to guarantee worms survival (reproduction factor 3 after 4 months in Deltares tests upon addition of straw). Nevertheless, the best result obtained throughout the preliminary tests of UofA was a factor 1 reproduction after 2 months and the presence of small worms (indicating new individuals). We now conclude that the lack of consistency in incubation period (2 months for the preliminary tests here, 4 months for Deltares) and in reproduction results resulted in a suboptimal design of the large-scale experiments containing straw. In fact, no worm was found alive after 4 months in the large-scale columns containing straw, which can be linked to the toxic conditions resulting from methanogenesis. It is hypothesized that for this type of tailings, the optimal in straw concentration that does not lead to methanogenesis and favors worm reproduction is to be found under 0.2% of straw, or perhaps for a different type of organic matter. On the other hand, 5 to 10% of the initial population of worms survived after 4 months in the large-scale column containing only TT and worms. This is smaller than what found in the Deltares tests for worms without amendment, and suggests that not all worms die naturally when added to tailings without other amendments, with the % of survival being a function of the type of tailing and having to be studied in depth prior to large scale testing.

Also in the large scale, there were no visible signs that *LV* worms went below the first 10-15 cm of the tailings in the large scale tests. On the other hand, we have differences in consolidation of almost 20 cm in absolute value with controls (therefore larger than the penetration depth as determined with the naked eye), and shear strength at the bottom of the columns (i.e. 1.2 m deep) was also larger than in controls, suggesting that the actual depth of influence of the worms cannot be estimated with the naked eye.

Summarizing, the technology has demonstrated a relevant performance when tested in thin layers of 30 cm under operational parameters (tailings type, solid contents, and temperature) given the measured enhanced permeability by the worms, and a mechanism to ensure the survival of the worms in the long term has been identified via the addition of straw at 3% in weight of dry solids and smaller. Incorporating this mechanism into the 30 cm experiments resulted in a similar performance of the technology and in worms reproduction, but not reaching the reproduction numbers that were expected, likely because adjustment of the mechanism for applying it at a somehow larger scale. In the final 1.85 m columns, the treatment of worms plus straw exhibited the largest consolidation, the largest strengths, and the largest solid contents among all large-scale tests. Improvements by the treatment decreased from what measured in the 30 cm layers, but the experiment was stopped before equilibrium and methanogenesis occurred due to sub-optimal design of the amount of straw for a new type of tailings. We presume that continuing the large-scale experiments until reaching equilibrium and properly designing the amount of straw as

a function of the tailings type to be treated will provide an actual quantification of the potential of worms to dewater 2 m layers of tailings and larger.

Multiple worms, some not applicable in Alberta, where tested in the small-scale tests, but the large-scale results where only performed for worms that could potentially be used in applications. Nevertheless, the research team does still strongly suggest to look for local possibilities of worms, and given economic and ecological considerations.

5.2 Recommendations for Future Work

In this section we will distinguish between two different type of recommendations. The first set of recommendations deals with the optimization of the technology and the mastering of the design of the optimal recipe, whereas the second set of recommendations deals with studies to evaluate the potential to apply the technology given its current state of development.

Recommendations on fine tuning of the treatment design:

- *LV* was introduced as a generic replacement for *Tubifex*, which was also an initial and generic worm choice, and whose objective was to prove that worm induced dewatering is relevant in Oil Sand tailings. Nevertheless, application is to be considered only for endemic species of worms. Therefore, a coherent next step would be to make a literature study of Oligochaete worms endemic to Alberta, selecting few candidates for being further studied in reproduction and survival tests.
- Once local species of *Oligochaete* worms has been identified, we can proceed to test their reproduction and survival for a relevant selection of tailings types. We strongly suggest to measure gradients in chemical and physical composition within one specific type of tailings (following Kamnisky's teams standard characterization of tailings at NAIT), considering the different results obtained for generic FFT and TT in Europe and North America (which were likely not that similar). In this way, we would be able to identify the tailings type and characteristics range that are worth of being treated with worms, eliminating the possibility of adding worms to a type of tailings were they will not develop. Possible amendments (like the addition of straw or any other type of cheaply available local organic waste) are also to be evaluated in this study.
- The large-scale tests were interrupted after 4 months, but were still not in equilibrium. We suggest to make large scale tests again, but this time wait until equilibrium is reached. Only then will be the potential of worms to act in thick layer (when added from the top) quantified.
- So far, we have only tested the technology in "static" beds. This means beds that are deposited at once and left to consolidate later, and where worms are added from the top. This is equivalent of adding the worms to a tailings pond that is to be closed. We could also consider adding the worms in beds that are still undergoing deposition (i.e. where the thickness of the tailings layer increases over time). We have recently collected evidence of worms surviving the placement of sediment-tailings at their interface: they migrate upwards towards the newly placed interface, tunneling the entire new layer and therefore extending their beneficial effect. This is likely to result in even better performances than the one recorded for the 1.85 m columns. We suggest to make a study to characterize the performance of worms under this conditions, as they will constitute a important alternative for the application of the technology.

- Tests in parallel research projects demonstrated that worms can help reaching 15 to 20 kPa under a 0.8 bar cap. Without worms, the exact same cap resulted in 0 to 5 kPa. And this was for an equivalent cap, but we foresee worm treated beds to withstand larger thicknesses of caps, following their enhance strength. Thus we suggest a study to quantify the potential improvements by capping and worms in tailings.
- In parallel research projects the effect of worms in unsaturated conditions (e.g. drying fields) has been quantified in 0.75 and 1.5 m layers and found to be vary from 45% to 65% in just few days, whereas in the absence of worms this takes 2 months. Moreover, Sc up to 70% are reached in equilibrium in worm treated beds, which is larger then the 60 to 65% Sc measured in controls. We suggest to explore the possibilities to enhance tailings desiccation with worms.

Recommendations on the evaluation of the technology's potential:

- Our partners at the TUDelft (Chassagne et al.), in collaboration with Deltares research staff, have developed a consolidation model following Gibson (and in some aspects equivalent to DELCON) that can be calibrated for mud without having to run the very laborious and expensive Seepage Induced Consolidation tests (Znidarčić). The only necessary condition is to have measured equilibrium bulk density profiles, which we have in this project. We thus suggest to use the data generated by the current project to calibrate such model and make predictions of the in-situ performance of the technology under several operational scenarios and applications.
- One of the clear application possibilities for this technology is capping. We therefore suggest to look for synergies with the current capping modelling research activities (led by BARR Eng.). We presume the strength measurements as performed in this study will be useful when modeling the possibilities for capping.
- Another important finding of our parallel research projects, is that worms can survive pumping. We still suggest to test worms pumping in the exact same pipes and pumps that will be used in the field, and prior to implementation.

5.3 Recommendations and challenges for application of the technology

Given the technical results in this study, a number of application possibilities has been identified, some of which require further research and preparatory work. These are:

- In a pond's closure, together with capping (see section 5.2 for further work recommendations in this field too).
- In a pond's closure, together with vegetation (subject of a on-going project for COSIA, in fact number 2018-14).
- In a pond that is still undergoing deposition (see section 5.2 for further work recommendations in this field too), and given the worms proven ability to survive deposition of sediment at their interface and to migrate upwards.
- In drying fields (see section 5.2 for further work recommendations in this field too).
- As indicated in the results of Task 1, FFT treated with only worms ends up having properties that are better (in particular strength and density, but not Sc) than this obtained after self-weight consolidation of TT. We therefore suggest to explore the possibility to densify FFT into the properties of TT before deposition in the pond, and as a green alternative for thickening plants.

Moreover, the research team has also identified a number of challenges related to the application of the technology. These are:

- The need to define the range of tailings properties in which the treatment can be beneficial.
- In some applications like on-going deposition, we would also prefer the worms to survive for economic reasons (then we only need to add worms once, at the lowest layer, and if they survive (or: thrive) they will keep on migrating through the subsequent deposited layers on top). A universal recipe or methodology to ensure worms reproduction is not yet optimized. For other applications like capping the worms survival is not as relevant, and we could see them as a temporary drain that stops being active after some time.
- A potential problem for this kind of reclamation might be lack of oxygen for worm survival under water cap. Only in shallow ponds where the mudline is not deep, simultaneous straw and worm amendment could increase tailings consolidation, as it is hypothesized that worms are best suited for tailings thickness where the surface is saturated with oxygen.

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7 REFERENCES

Elissen, H. J. H. (2007). Sludge reduction by aquatic worms in wastewater treatment, with emphasis on the potential application of. Lumbriculus variegatus.

Hendrickx, T. L. G., Elissen, H. J. H., Temmink, B. G., & Buisman, C. J. N. (2007). Een nieuw reactorconcept voor slibreductie met aquatische wormen. Afvalwaterwetenschap, 6(1), 45-52

Clifford, H. F. (1991). Aquatic invertebrates of Alberta: An illustrated guide. University of Alberta.

Davis RB (1974) Stratigraphic effects of tubificids in profundal lake sediments. Mar Eco Prog Ser 19(1974):466–488

de Lucas Pardo, M. A., Bakker, M., van Kessel, T., Cozzoli, F., & Winterwerp, J. C. (2013). Erodibility of soft freshwater sediments in Markermeer: the role of bioturbation by meiobenthic fauna. Ocean Dynamics, 63(9-10), 1137-1150.

Yang, X., de Lucas Pardo, M., Ibanez, M., Deng, L., Sittoni, L., Ulrich, A., & Chen, Q. (2020). Improving engineering properties of mature fine tailings using Tubifex. Canadian Journal of Civil Engineering, 47(7), 812-821.

An, D., Caffrey, S.M., Soh, J., Agrawal, A., Brown, D., Budwill, K., Dong, X., Dunfield, P.F., Foght, J., Gieg, L.M., Hallam, S.J., Hanson, N.W., He, Z., Jack, T.R., Klassen, J., Konwar, K.M., Kuatsjah, E., Li, C., Larter, S., Leopatra, V., Nesbø, C.L., Oldenburg, T., Pagé, A.P., Ramos-Padron, E., Rochman, F.F., Saidi-Mehrabad, A., Sensen, C.W., Sipahimalani, P., Song, Y.C., Wilson, S., Wolbring, G., Wong, M.L., Voordouw, G., 2013. Metagenomics of hydrocarbon resource environments indicates aerobic taxa and genes to be unexpectedly common. Environ. Sci. Technol. 47, 10708-10717.

Dong X., Kleiner M., Sharp C.E., Thorson E., Li C., Liu D. and Strous M. 2017. Fast and Simple Analysis of MiSeq Amplicon Sequencing Data with MetaAmp. Front. Microbiol. 8, 1461.

Lou, K., Rajapaksha, A.U., Ok, Y.S. et al. 2016. Sorption of copper(II) from synthetic oil sands process-affected water (OSPW) by pine sawdust biochars: effects of pyrolysis temperature and steam activation. J Soils Sediments 16, 2081–2089

Pourrezaei P., Alpatova A., Khosravi K., Drzewicz P., Chen Y., Chelme-Ayala P., Gamal El-Din M. 2014. Removal of organic compounds and trace metals from oil sands process-affected water using zero valent iron enhanced by petroleum coke. J Environ Manag 139, 50–58

Scott, A.C., Zubot, W., MacKinnon, M.D., Smith, D.W., Fedorak, P.M., 2008. Ozonation of oil sands process water removes naphthenic acids and toxicity. Chemosphere 71 (1), 156–160.

SESDPROC-113-R2, Field Measurement of Oxidation-Reduction Potential (ORP). USEPA, 2017

Siddique, T., Kuznetsov, P., Kuznetsova, A., Arkell, N., Young, R., Guigard, S., Foght, J.M., 2014b. Microbially-accelerated consolidation of oil sands tailings. Pathway I: changes in porewater chemistry. Front. Microbiol. 5, 106.

Siddique, T., Kuznetsov, P., Kuznetsova, A., Li, C., Arocena, J.M., Foght, J.M., 2014a. Microbially-accelerated consolidation of oil sands tailings. Pathway II: changes in solid phase chemistry. Front. Microbiol. 5, 107.

Węgrzyn G., Czyż A. 2002. How do marine bacteria produce light, why are they luminescent, and can we employ bacterial bioluminescence in aquatic biotechnology? Oceanologia 44, 291-305.

Amaro, T.P.F., Duineveld, C.A., Bergman, M.J.N., Witbaard, R. and Scheffer, M., 2007. The consequences of changes in abundance of Callianassa subterranea and Amphiura filiformis on sediment erosion at the Frisian Font (south-eastern North Sea. Hydrobiologia, 589, 273-285.

Brinkhurst, R.O., 1978. Freshwater Oligochaeta in Canada. Canadian Journal of Zoology, 56(10), 2166-2175.

Chapman, P., 2001. Utility and relevance of aquatic Oligochaetes in Ecological Risk Assessment. Hydrobiologia, 463, 149-169.

Davis, R. B., 1974. Stratigraphic effects of tubificids in profundal lake sediments. Limnol. Oceanogr, 19, 466-48.

Davis, R. B., 1974. Tubificids alter profiles of redox potential and pH in profundal lake sediment 1. Limnology and Oceanography, 19(2), 342-346.

de Lucas Pardo, M.A., Bakker M., van Kessel T. , Cozzoli F. and Winterwerp J.C., 2013. Erodibility of soft freshwater sediments in Markermeer the role of bioturbation by meiobenthic fauna. Ocean Dynamics, 63 (9-10), 1137-1150.

de Lucas Pardo, M.A., 2014. Effect of biota on fine sediment transport processes. A study of Lake Markermeer. Ph.D. thesis, Department of Environmental Fluid Mechanics, Delft University of Technology, Delft, the Netherlands.

Engle, V.D., Summers, J.K. and Gaston, G.R., 1994. A benthic index of environmental condition of gulf of Mexico estuaries. Estuaries, 17(2), 372-284.

Huang, X., Liang, P. and Qian Y., 2007. Excess sludge reduction induced by Tubifex Tubifex in a recycled sludge reactor. Journal of Biotechnology, 127(2007), 443-451.

Liang, P., Huang, X., Qian Y., Wei, Y. and Ding, G., 2006. Determination and comparison of sludge reduction rates caused by microfaunas' predation. Bioresource Technology, 97(2006), 854-861.

Lucan-Bouché, M. L., Biagianti-Risbourg, S., Arsac, F. and Vernet, G., 1999. An original decontamination process developed by the aquatic oligochaete Tubifex Tubifex exposed to copper and lead. Aquatic toxicology, 45(1), 9-17. Merckelbach, L. M., & Kranenburg, C. (2004). Equations for effective stress and permeability of soft mud–sand mixtures. Géotechnique, 54(4), 235-243.

Rhoads, D.C. and Young, D.K., 1970. The influence of deposit-feeding organisms on sediment stability and community trophic structure. Journal of Marine Research, 28(2), 150-178. Whiting, E. R. and

Clifford, H. F, 1983. Invertebrates and urban runoff in a small northern stream, Edmonton, Alberta, Canada. Hydrobiologia, 102(1), 73-80.

Widdows, J., Brinsley, M.D., Bowley, N. and Barrett, C., 1998. A benthic annular flume for in situ measurement of suspension feeding/biodeposition rates and erosion potential of intertidal cohesive sediments, 46, 27-38.

Widdows, J., Brinsley, M.D., Salked, P.N. and Lucas, C.H., 2000. Influence of biota on spatial and temporal variation in sediment erodability and material flux on a tidal flat (Westerschelde, The Netherlands). Marine Ecology Progress Series, 194, 23037.

Willows, R.I., Widdow, J. and Wood, R.G., 1998. Influence of an infaunal bivalve on the erosion of an intertidal cohesive sediment: A flume and modeling study. 43(6), 1332-1343.

Yang, X., de Lucas Pardo, M., Deng, L., Ulrich, A., Sittoni, L., and Chen, Q. 2015. Tubifex: A biological method for dewatering oil sands tailings. Tailings and Mine Waste 2015 Proceeding, 499 – 507.

Yang, X., de Lucas Pardo, M., Deng, L., Sittoni, L., Ulrich, A., and Chen, Q. 2016. Accelerating dewatering of oil sand tailings using laboratory Tubifex and Polymer-Tubifex treatments. (in preparation). Institute for Oil Sands Innovation June 2016 11

Zhu, H., Wei, Y. S. and Wang, Y. W., 2008. Effect of Tubificidae on activated sludge settling performance. Acta Scientiae Circumstantiae, 28(5), 910.

APPENDIX: LIST OF PUBLICATIONS AND PATENT FILING/APPLICATION

Under preparation: Optimizing the usage of LV worms in Oil Sand Tailings. Tio be submitted to the Canadian Journal of Civil Engineering. We are still currently considering whether to do two or one paper out of the results in this report: small scale and large-scale tests. Two previous papers from our team were already published in the Canadian Journal of Civil Engineering.