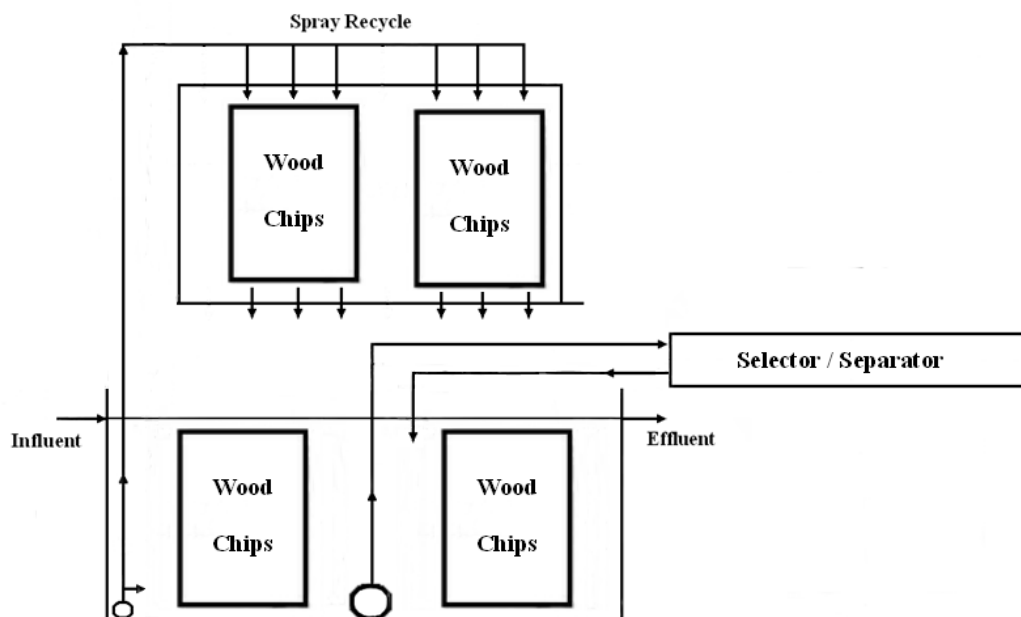


Appendix B: TF1 Application.

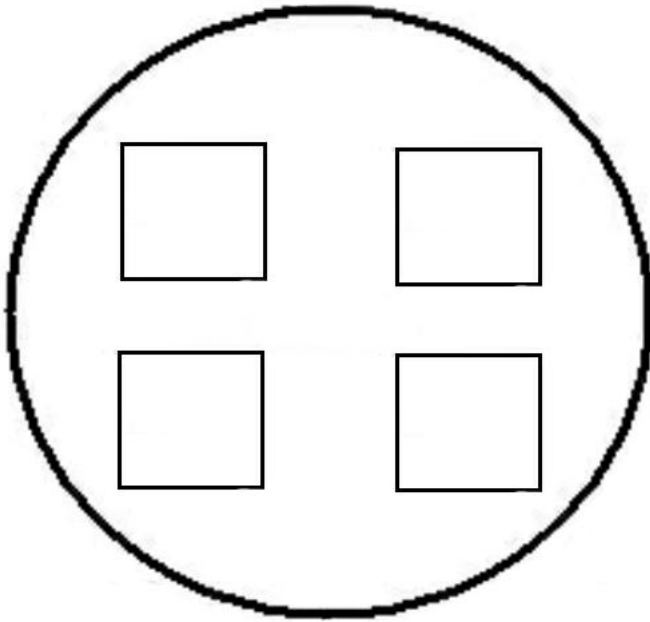
Here is an example of how the general specifications can be applied to a TF1 batch mode.

A typical TF1 system is contained in and above a 1,000 gallon circular tank that is 8 feet in diameter and 31 inches tall. There is a 4 foot by 4 foot by 3 foot basket perched above the tank which contains wood chips. These wood chips are contained in four 22 inch by 22 inch by 30 inch smaller baskets placed within the larger basket. All of the four smaller baskets are irrigated with water from the tank which flows through the wood chips and back into the tank below. There are other small wood chip baskets with the same dimensions submerged in the tank and a 100 gallon two part solids collector/ organism selector that can be located next to the in tank cube system. The system requires a water source and drain, electrical power, aeration, pumps, and a support structure to provide access to all components of the system. It also comes with a test kit and instruction manual. The 1,000 gallon tank can be buried in the ground or placed at ground level.

Here is a diagram of a typical TF1 unit.



Here is a diagram is looking straight down at the tank with submerged chip baskets.



Each of the submerged and irrigated small chip baskets will have a volume of 63 gallons and will hold wood chips at a density of 2 pounds per gallon. Thus there will be a total of 504 pounds of wood chips in both the submerged and irrigated baskets for a total wood chip loading of 1,008 pounds, dry weight.

In this example the water volume in the tank will be 940 gallons. Subtracting the 252 gallons taken up by the wood chip baskets leaves 688 gallons of free water volume for the fish. Assume that the system will operate with drinking water with a 100 day Hydraulic Retention Time. Thus between 7 to 10 gallons of drinking water will be added to the system per day resulting in an effluent of 7 gallons per day with some evaporation from the system. Since ammonia and phosphorus are not normally regulated in drinking water we will assume a tertiary treatment wastewater effluent water quality standard of 2 mg/ l for Total Ammonia Nitrogen with phosphorus at about 0.5 mg/l for our influent water.

At a basic level we will operate with only test kit measurements for pH, temperature, salinity, ammonium nitrogen and orthophosphate. Thus we will keep pH at 7.0 or lower to ensure a low ammonia (NH_3) to ammonium ion (NH_4^+) ratio

so that our ammonium nitrogen measurements will reflect the bulk of the N available. An ammonium ion to orthophosphate ratio will be maintained at 4 to 1.

For the initial run of the batch process load the tank with water and the baskets with wood chips. Once the species of fish are selected, rainbow trout in this example, obtain inoculum sediment and water samples from local lakes, streams, and wetlands for microorganisms and desired invertebrates. For this case the invertebrates should include terrestrial earthworms, aquatic oligochaete worms, and freshwater snails. Local insects and insect larvae are also candidates if they are easy to cultivate within the system. Small minnows may be useful when larger fish are developed.

Run the recycle and aeration systems for a week or two to ensure that aeration and recycle pumping systems are operating smoothly. Then introduce 22 fingerling trout weighing about one ounce each, for an initial stocking weight of 22 ounces. The plan is to run the system for 8 months and then to harvest 20 trout weighing about one pound each. This will occur when the fish biomass density reaches 0.029 pounds of fish per gallon of available water. We expect that there will be two morts, one weighing 4 ounces and a second weighing 8 ounces. Thus at 8 months we will have produced 20.75 total pounds (332 ounces) of fish, containing 0.415 pounds (6.64 ounces) of N, with 20 pounds of fish being harvested consumable product.

We will feed the trout during this first batch process to build up the microbial and invertebrate populations as well as grow the fish. Following the general guidance from the Evaluation Study performed at the Freshwater Institute in 2010 and 2011 (attached) we project that hand feeding the fish on a daily basis to no more than satiation will result in a Specific Growth Rate of about 2.5 percent per day yielding a doubling time for total fish weight every two months.

The feed we will use is a high protein trout chow with 48 percent protein. The Freshwater study reported a Feed Conversion Ratio (FCR) of 0.7 so producing 20.75 pounds of fish would require 14.525 pounds of feed containing about 1.162 pounds (18.59 ounces) of N. But the total fish produced (20.75 pounds or 332 ounces) contained about 0.415 pounds (6.64 ounces) of N. This leaves 11.95 ounces of N from the feed still in the system or discharged in the effluent.

There will be some nitrogen in the wood and we will assume that this will be 0.05 percent on a dry weight basis. This will add an additional 0.504 pounds or 8.06 ounces of Nitrogen to the 18.59 ounces from the feed. Subtracting the nitrogen

taken up by the fish will leave a total amount of N remaining in the system after the fish will then be 20 ounces.

The effluent of 7 gallons per day with 2 mg/l of N discharges 0.0001168 pound of N per day or 0.000187 ounces per day. Over 8 months this amounts to less than 0.05 ounces of N. Thus 11.9 ounces of N stay in the system after 8 months.

This N will be distributed over the microbial and invertebrate populations remaining in the system. Since it takes about 4 pounds of microbes to produce one pound of invertebrates we assume that this N distribution will be 80 percent in the microbes and 20 percent in the invertebrates. Thus in the 20 ounces of N staying in the system 16 ounces will be in the microbial biomass.

Harvesting the wood chips will be done in a manner that removes all invertebrates and most of the microbial biomass from the chip. Some of the microbes will stay attached to the harvested chip and some of them will be settled out and collected in the solids collector/separator. If 10 percent of the total microbial solids produced are removed this way that would subtract another 1.6 ounces from the system, and this would leave about 18.4 ounces of N in the active system.

So for the 18.59 ounces of N that were introduced to the first batch as fish feed, and the 8.06 ounces of N that came from the wood, we subtract the 0.05 ounces that left in the effluent, and the 6.64 ounces that were removed in the fish, and 1.6 ounces that left in the removed settled solids. This leaves 18.36 ounces of N still in the active system after the first batch run.

So now we bring our wood chip inventory back up to 1,008 pounds and introduce another 22 fingerling trout. What do we need to add as feed if we will harvest 20 trout at one pound each, have two morts as before, and leave the system in the same condition at the end of the second batch run as it is at the end of the first batch run. Since we already have 18.36 ounces of N in the system we should only need to add $18.59 - 18.36$ or 0.23 ounces of N in added new feed. This would be about 3 pounds of new feed for the second run compared with 14.525 pounds of feed for the first run. These could be fed as needed over the second 8 months of operation. Thus for every run after the first, the system would only need 3 pounds of feed containing 0.23 ounces of N to produce 20 pounds of final product fish. Conventional operations using a FCR of 1.0 would require 22.75 pounds of feed per each run.

Note that in this run we used a target fish biomass density of 0.029 pounds of fish per gallon of water. Raising this number to 0.1 pounds of fish per gallon (10 gallons of water for each pound of fish) would triple the output of this system. Also note that if the wood chips had a nitrogen content of 0.1 percent (instead of 0.05 percent) that no additional feed would be required after the first run.

The TF1 unit is basically an indoor unit in that it needs protection from freezing temperatures and other environmental impacts. The unit can be located in a garage, barn, laboratory, green house or comparable structure. In temperate climates or for seasonal operation units can be located outdoors where they can be a part of ornamental gardens or parks.

If space is limiting a smaller 500 gallon version is available. This can show structure, process, and fish raising ability, but is generally too small to demonstrate quantifiable fish production capability.

Here are some photos of a TF1 system. These photos have a single chip basket in a four foot cube perched above the fish tank instead of the four smaller chip baskets described above.



