

Removal of Nitrogen by Bioreactor Method

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Abstract Excessive nitrogenous compounds released into the public water bodies not only result in direct toxicity to aquatic animals, but also increase the overgrowth of aquatic plants resulting eutrophication. Nitrogen pollution has major effects on both human health and the ecological functions of natural ecosystems. This causes a spike in algae growth, which can rapidly deplete the dissolved oxygen in a body of water, causing harm to fish and the surrounding ecosystem. For this reason, it is necessary to study the process of nitrogen removal in wastewater treatment plants in order to remove nitrogen efficiently. The processes of nitrification and denitrification in a sequencing batch reactor. A nitrate test kit and spectrophotometer in order to measure nitrate concentrations throughout the various stages of our reactor cycles. Initially found it difficult to achieve nitrification in our plant. After increasing the dissolved oxygen levels during our aeration stage, nitrate began to be formed. In order to achieve higher levels of nitrification, it was necessary to increase the length of the aeration stage. It was also necessary to increase the length of the anaerobic phase in order to allow for significant denitrification.

Keywords: Bioreactor, Suspended Solid, Oxidization, Sludges

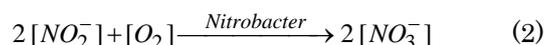
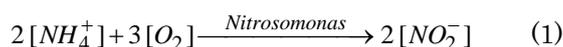
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1. Introduction

Nitrogen (N) is one of the nutrients essential to living organisms. Microbial reactions drive the global nitrogen cycle and the process of nitrification is central to it. Human activities have drastically impacted the global nitrogen cycle [1]. The excess of nutrients causes algae to flourish, depleting the oxygen levels in the water and killing fish or other aquatic animals. There are also certain human health concerns associated with the eutrophication of lakes, such as the spread of *Escherichia coli* [2]. Algae blooms are aesthetically unpleasing since they can cause foul odors and spoil beaches and shorelines. Humans contribute to the addition of excessive amounts of nitrogen, phosphorus, and carbon in many ways. Some major sources of effluent include runoff from agricultural fields, golf courses, and urban lawns, in addition to untreated or partially treated sewage effluent. In order to prevent the occurrence of lake eutrophication, it is a priority for wastewater treatment facilities to remove these nutrients from the sewage prior to discharge into receiving waters [3]. Nitrogen removal can be accomplished by several methods. Nitrogen removal in activated sludge processes can be done by assimilation into new cell tissue and subsequent sludge wasting, but in most municipal and many industrial wastewaters, excess nitrogen exists that cannot be removed by conventional treatment [4,5,6,7]. In cases of excess, nitrogen may be removed by nitrification followed by denitrification where nitrogen is released in the form of nitrogen gas. This significantly decreases the concentration of nitrites and nitrates in the effluent, which

can be the limiting reagent for eutrophication in many shallow lakes and estuaries. More than 90 % of the nitrogen in raw domestic wastewater is in the form of ammonia or compounds from which ammonia is readily formed [8,9,10]. With proper environmental conditions during treatment, the ammonia will be converted to nitrate. The nitrogen compounds found in raw sewage may be biologically oxidized provided that the proper environment is maintained in a biological treatment process. When nitrogen-containing organic material is oxidized, ammonia-valence nitrogen is released into solution. This nitrogen is available as a nitrogen and energy-source for the nitrifying bacteria, posing as an oxygen-demand threat to receiving waters [11]. Nitrifying bacteria, however, converts these nitrogen compounds to nitrites (NO_2^-) and nitrates (NO_3^-). The nitrogen cycle is shown Figure 1.

There are two groups of nitrifying bacteria: *Nitrosomonas*, which oxidizes ammonia nitrogen to nitrite, and *Nitrobacter*, which oxidizes nitrite to nitrate [24]:



There are certain conditions that are necessary to carry out nitrification in the activated sludge process. The pH level should be between 6.5 and 8 and the temperature should be around 25° Celsius in order to achieve optimal levels of nitrification. For every 10° Celsius the temperature increases, the nitrifier growth rate doubles. There must also be a significant amount of dissolved oxygen of at least 2 mg / L in the system. Certain

compounds such as mercury, zinc, and ethanol can inhibit the nitrification process, so care must be taken that these compounds are not present in the sludge reactor. Denitrification occurs in the anaerobic stages of the treatment process. The organic cells use nitrogen as the electron acceptor when oxygen is not present, so dissolved oxygen levels above 0.3 mg / L will inhibit the denitrification process (Culp 397). During denitrification, nitrate or nitrite is reduced to nitrogen gas, which is then released into the atmosphere [12]. This step decreases the amount of nitrate and nitrite that would normally be found in the effluent of untreated activated sludge.

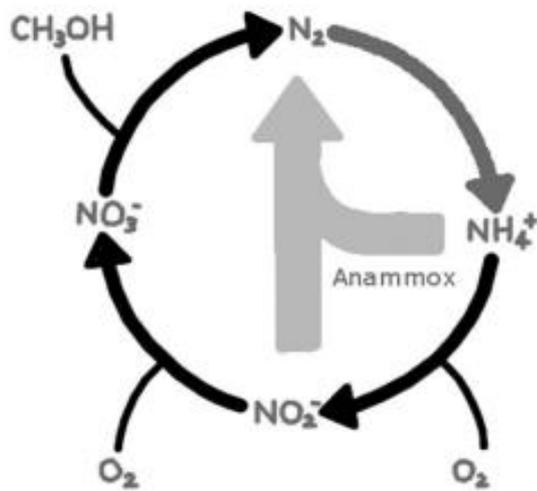
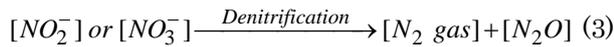


Figure 1. Nitrogen cycle

In large-scale systems, the wastewater is moved into a secondary clarifier for the anaerobic stage where the residence time system is extended to at least two or three days long and an organic carbon source is added. The source of available carbon greatly affects the rate at which denitrification occurs [10,13]. The highest rate will be achieved by an easily assimilated carbon source such as methanol, but this is costly to purchase and store. The addition of raw sewage will act as an effective carbon source, but the denitrification rate will be lowered. In addition, denitrifiers are generally less sensitive to inhibitory compounds than nitrifiers [14,15]. In this regard an effort has been made to examine the effectiveness of nitrogen removal by batch reactor. Design reactors for optimum removal of nitrogen compare the total nitrogen removal for two different reactor configurations. The first configuration was to model a single aeration stage followed by a single anaerobic stage, and the second was a modified version of the Barnard Process.

2. Materials & Methods

2.1. Experiment Setup

The traditional Barnard Process begins with an anaerobic stage to allow for denitrification to occur [16]. In this stage, synthetic waste serves as the primary electron donor for conversion of nitrate to nitrogen gas. This stage is then followed by a long aeration phase, and then another anaerobic phase. In this second anaerobic phase, biomass (i.e. the bacterial cells) serves as the primary electron donor for further denitrification. Another short aeration phase then follows.

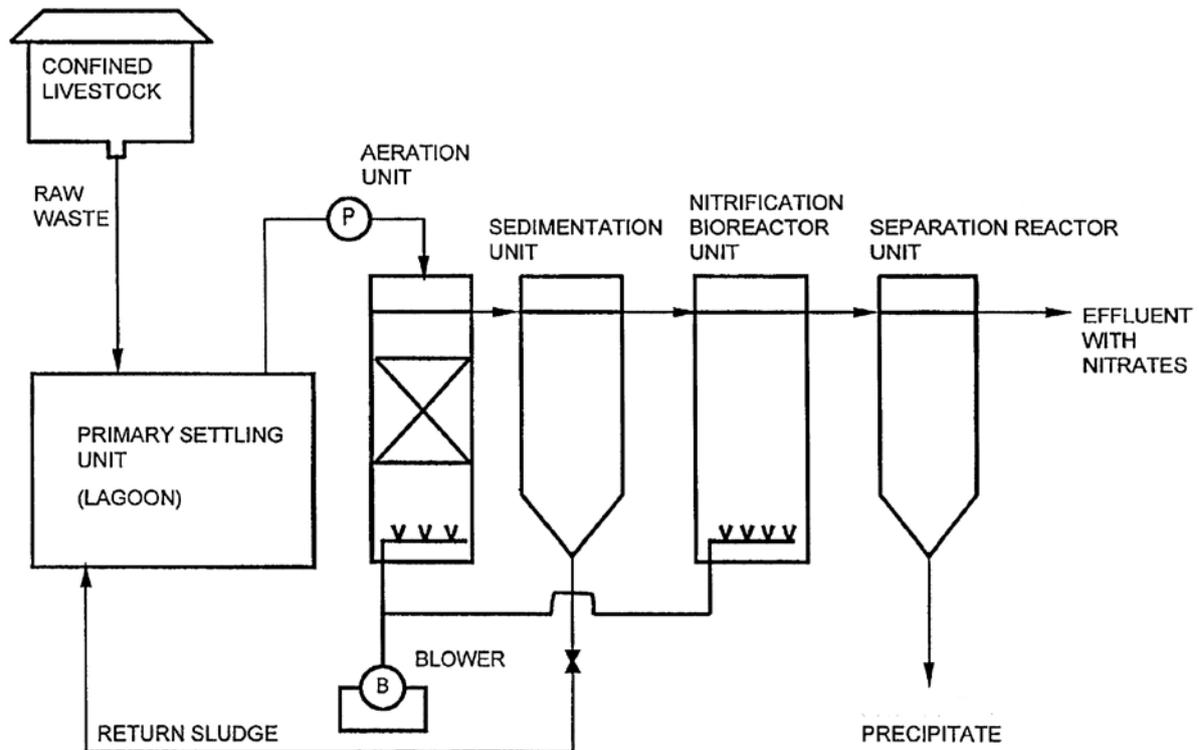


Figure 2. Barnard Process

As modified version of the Barnard Process, we intended to begin the process with an extra aeration phase

in order to allow for initial nitrate formation. It's shown above in Figure 2, when the Barnard Process is carried out

in a continuous flow reactor, some of the effluent from the first aeration step is recycled back to the first anaerobic reactor. Since nitrification occurs in the aeration process,

the result is the recycling of some nitrate back to the first step, allowing for a better breakdown of nitrogen.

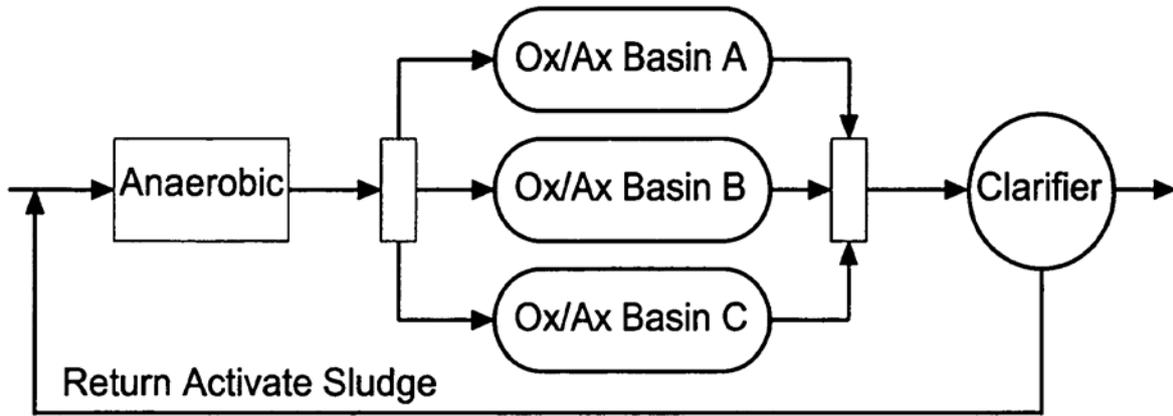


Figure 3. Barnard Process in a Sequencing Batch Reactor

In Figure 3, when the Barnard Process is modeled in a sequencing batch reactor, none of the waste in the initial anaerobic stage will have undergone an aeration phase (i.e. nitrification), since there is no sludge recycle from the previous process. Hence, there would likely be a low concentration of nitrate in this first anaerobic stage, and therefore little denitrification, since our incoming organic waste contains nitrogen mainly in the form of ammonium. For this reason, it was decided to add an additional aeration stage in the beginning of the process. This stage would hopefully allow for some nitrification to occur, but would not be long enough to break down all of the incoming waste, leaving some of the waste to serve as the

electron donor for denitrification in the following anoxic stage. The second configuration will utilize biomass storage and decay. This process will begin with an aeration phase in which nitrification can occur and the BOD can be depleted. Phase will then be followed by an anaerobic stage, which will allow for denitrification to occur. The primary electron donor will be our biomass. Figure 4 shows an outline of this process for a traditional treatment plant. Since we will be employing a sequencing batch reactor, most of the waste will exit with the effluent, while the bulk of the biomass (cells) will remain at the end of the cycle.

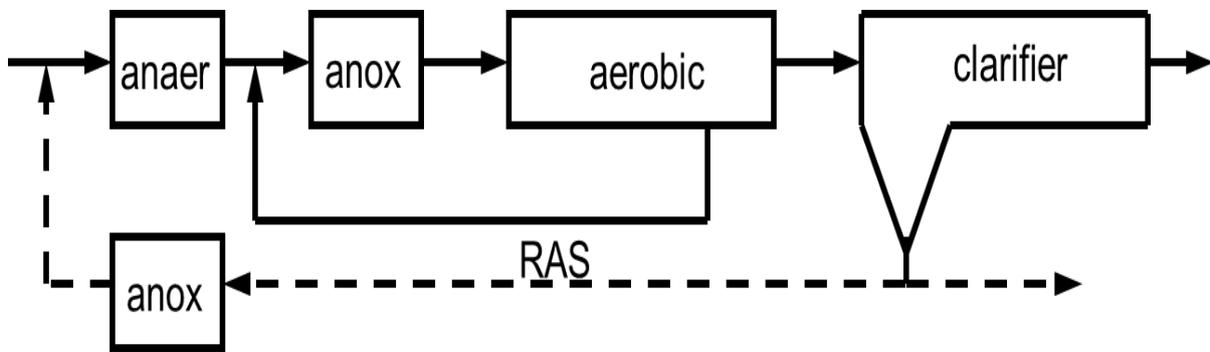


Figure 4. Biomass Storage & Decay

2.2. Control Parameters

The Process Controller software (v2.0) to control the reactor was used. In Table 1 it shows the list of states for reactor, along with the exit condition for each.

The overall residence time for our reactor was 6 hours. Since it was unable to employ the Barnard Process, the control logic shown in Table 1 is the same logic used throughout entire experiment. However, vary the length of certain set points throughout experiment. The initial values for set points are shown below in Table 2.

Table 1. States and exit conditions for our sequencing batch reactor

State	Exit Condition
Add organic feed	Elapsed time > pump time for organic feed
Fill with water 1	Reservoir level > max reservoir level
Aerate	Elapsed time > max aeration time
Stir	Elapsed time > max stir time
Settle	Elapsed time > max settling time
Drain	Reservoir level < min reservoir level

Table 2. Values for control logic set points

Set Point	Initial Value	Final Value
Max aeration time (seconds)	8700	13,800
Max stir time (seconds)	8700	3600
Max settling time (seconds)	3600	3600
Max reservoir level (mL)	4000	4000
Min reservoir level (mL)	1000	1000

As Table 2 shows, for the latter stage of experiment, we increased the duration of the aeration phase and decreased the duration of the anaerobic (stirring) phase. This was done in an effort to increase nitrate formation in the reactor, while keeping the hydraulic residence time the same. For both setups, the hydraulic residence time remained 6 hours. A peristaltic pump is added both the organic waste and the water to reactor. A pressure sensor was used in order to measure the level of water in the reactor in order to determine when to stop adding water and to stop draining. Using a dissolved oxygen probe to measure the DO concentration; data for DO concentration was continuously logged to a computer file while the plant was in operation. The rubber end of the stirring rod was used to clean the sides of the tank. It was used in a spatula-like manner to feverishly scrape the sludge. Rubber gloves were worn throughout this procedure - in a manner similar to that of wearing oven mitts while baking. A paper towel was sometimes draped over the top of the reactor in order to keep sludge from splashing onto the tabletop.

2.3. Nitrate Testing

For experiment, Nitrate Test Kit was used. This kit uses cadmium and other reagents to determine nitrate concentrations [17]. When these reagents are added to solutions containing high concentrations of nitrate, the solution turns a deep shade of red. The intensity of the red color corresponds to the nitrate concentration, which can be determined using either the color viewing apparatus supplied with the test kit or a spectrophotometer. UV

Spectrophotometer was used to determine the nitrate concentration for treated samples. Standards were prepared with concentrations ranging from 0 to 7 mg / L nitrate nitrogen. All of the samples from our plant were compared against these standards using the nitrate test kit and spectrophotometer. The sample concentrations were determined using the absorbance at 542 nm. All of the samples were taken from the top of the reactor, allowed to settle for at least 15 minutes, and then filtered before performing the tests. Since our highest standard concentration was 7 mg / L, all of our samples were diluted until the spectrophotometer measured concentrations of 10 mg / L or less, in order to minimize error due to extrapolating. We attempted to analyze our samples without the use of the nitrate test kit, according to the procedure provided on the CEE 453 website (Weber-Shirk). For this procedure, we created 50 mL standards ranging from 0 to 10 mg / L nitrate nitrogen, and added 1 mL of 1 N HCl to each sample. These samples were then analyzed using the spectrophotometer, and the greatest correlation was observed at a wavelength of 220 nm (r value = 0.9999). We then analyzed our filtered samples in the spectrophotometer (adding only HCl) and compared the absorbance values against our standards in order to determine a value for the nitrate nitrogen concentration.

3. Results & Discussion

3.1. NNC (Nitrate Nitrogen Concentrations) First Stage

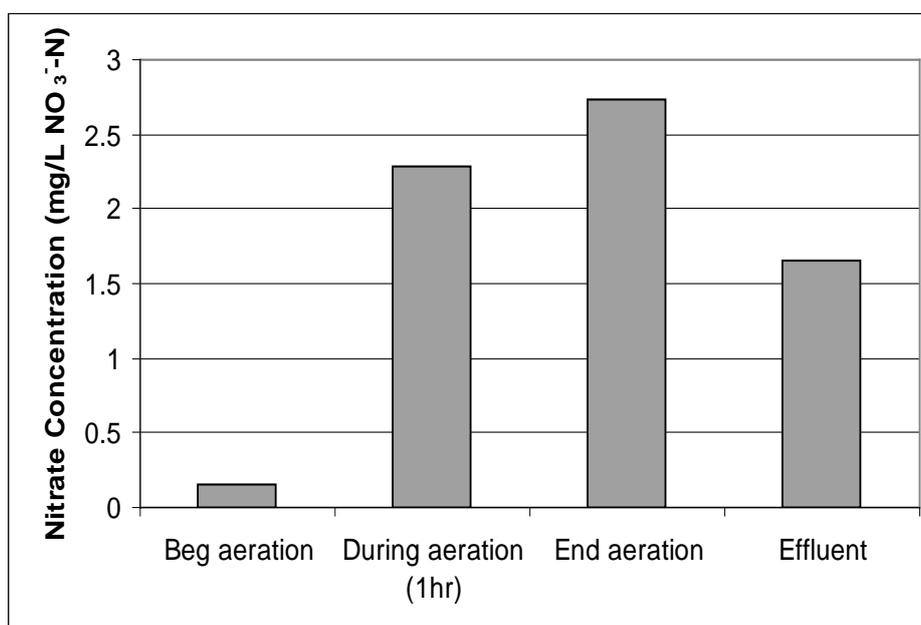


Figure 5. Nitrate nitrogen concentrations for initial reactor configuration. Aeration time was 8700 seconds; anaerobic stirring duration was 8700 seconds

To determine the nitrogen removal reactor was initially set to aerate for 8700 seconds and to stir without aerating for another 8700 seconds. After operating in this manner for over two weeks, we took samples in order to measure the nitrate concentration. The nitrate test was performed on samples taken throughout the entire process, from the addition of the synthetic waste until the effluent was drained. The resulting nitrate nitrogen concentrations (using the test kit) were minimal. Not a single sample turned red, and the spectrophotometer returned negligible (or even negative) nitrate nitrogen concentrations at a wavelength of 542 nm [18]. The unexpected results led us to rethink objectives. Before it could perform any sort of analysis on the effectiveness of nitrogen removal, first make our reactor conducive to the nitrification-denitrification process. The pH of the reactor was around 8.6, which is within the optimal range for both nitrification and denitrification. The temperature of reactor was about 21°C, which is slightly lower than the optimal 25°C, but not low enough to completely inhibit nitrification [19]. The resulting concentrations are shown in Figure 5.

3.2. NNC (Nitrate Nitrogen Concentrations) Second Stage

To determine the dissolved oxygen concentration. The DO concentrations had initially started out high during the aeration phase for the first few weeks that our reactor was running. But as the reactor ran longer, the air flow rate seemed to be dropping, and the DO measurements confirmed that our DO level was rarely exceeding 4 mg / L. This was probably due in part to the aeration stone becoming clogged with sludge. The low DO level to be what was inhibiting nitrification, so increased the air flow rate and closely monitored the DO levels to make sure they stayed above 5 mg / L. After the reactor ran through a few cycles aerating at high DO levels, we again tested the nitrate levels through the various stages of the cycle. The increase in DO levels seemed to stimulate the start of the nitrification process. Now nitrate formation was more in the reactor, it was focus how to make it more effective. Adding at least 40.9 mg / L nitrogen to the reactor (in our synthetic waste), NO_3^- -N levels of 2.75 mg / L at the end of aeration (Figure 4) mean that complete nitrification was not taking place. Since the increase in DO levels during the aeration phase seemed to have helped, It was decided to run the aeration phase for a longer period of time to see what nitrate levels could be achieved. The total hydraulic residence time of our reactor the same and simply increased the total aeration time by decreasing the amount of time spent in the anaerobic stage. The resulting nitrate nitrogen concentrations are shown in Figure 6.

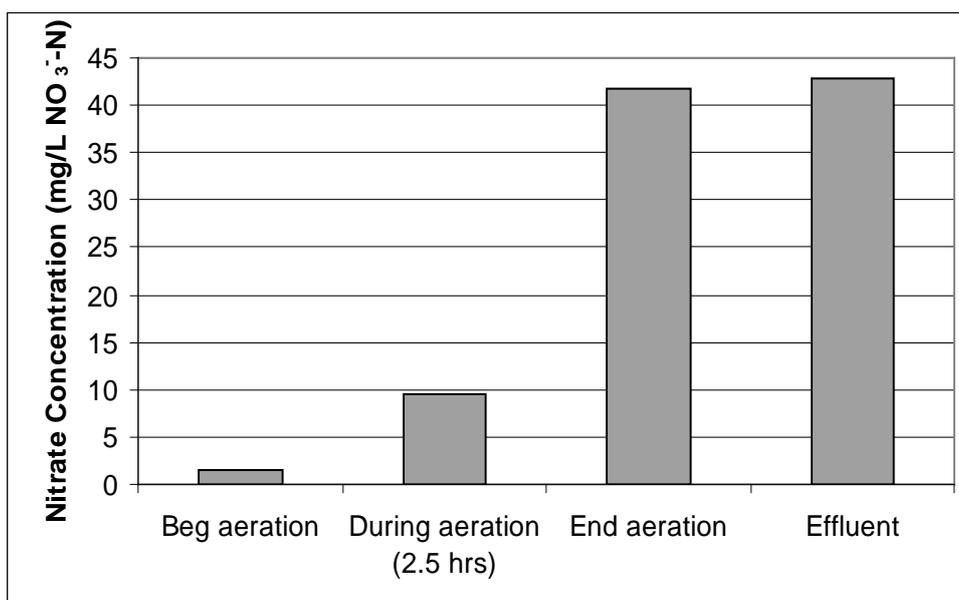


Figure 6. Nitrate nitrogen concentrations for second reactor configuration. Total aeration time was 13,800 seconds; total anaerobic stirring duration was 3600 seconds

It is clear from Figure 6 that the increase in total aeration time significantly benefited the nitrification process. By the end of 13,800 seconds of aerating, we had achieved nitrate nitrogen levels of 41.8 mg / L. However, since we only allowed the reactor to stir without oxygen for 3600 seconds, there was not enough time for denitrification to occur. In fact, the measured nitrate nitrogen concentration in our effluent (after denitrification) was even higher than was measured at the end of aeration [20]. This is likely due to the error in determining the concentration; however, it is not encouraging since nitrate levels should be depleted during the denitrification process.

3.3. NNC (Nitrate Nitrogen Concentrations) Final Stage

To determine the current setup of reactor, it would be very difficult to achieve total nitrification and denitrification and still maintain the residence time of 6 hours. We decided to set the reactor to aerate for a very long time (18 hours), and then stir anaerobically for another 11 hours. The concentrations throughout the process and the results are shown below in Figure 7.

From results, it was concluded that both the processes of nitrification and denitrification seem to be occurring relatively slowly, and for our sequencing batch reactor it is

difficult to achieve complete nitrogen removal with a residence time of 6 hours. It was found to be very important to keep the dissolved oxygen concentration very high (above 5 mg / L) during the aeration phase in order to achieve nitrification. It is possible that the nitrification process could be accelerated by heating the reactor contents to the optimum temperature for nitrification at 25°C [21]. The denitrification process might be occurring slowly because we are using the cell biomass as the

electron donor. This might explain why the process appears to have stalled after 4-hours; that is, perhaps the biomass took all the electrons it could, and caused the denitrification process to stop. Denitrification could likely be speeded up by adding some sort of additional organic electron donor (such as methanol), or perhaps by configuring the reactor to use the incoming waste as an electron donor (as we had planned to, using our modified Barnard Process) [22].



Figure 7. Nitrate nitrogen concentrations for final experiment. Total aeration time was about 18 hours; total anaerobic stirring duration was about 11 hours

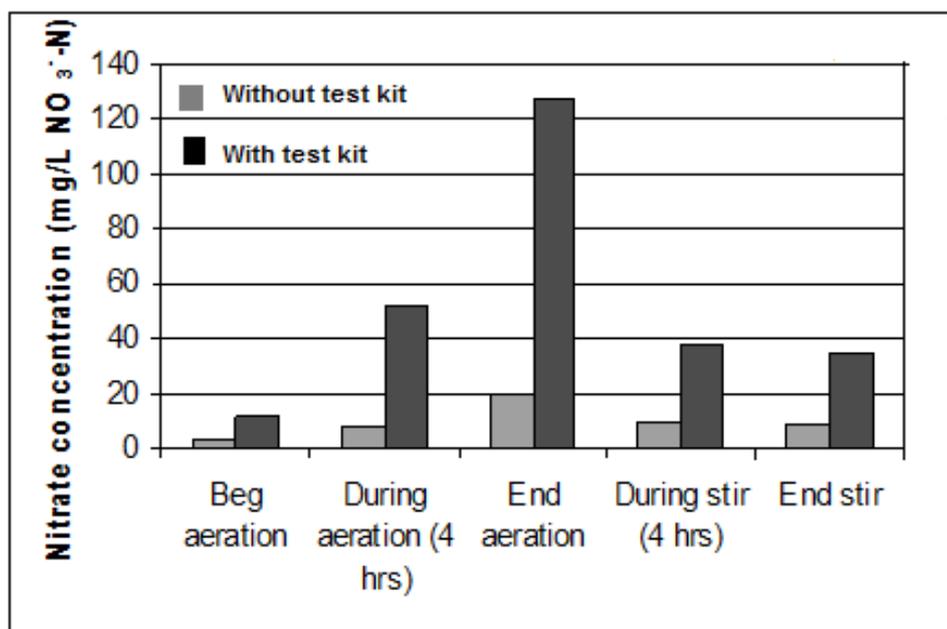


Figure 8. Nitrate nitrogen concentrations for the same test from Figure 6. Results given for measured concentrations using the test kit (same as Figure 6) and without using the test kit

3.4. Comparatively Studied

The objective of this experiment was to determine the feasibility of the alternate process of determining nitrate concentration. This method involves analyzing the

absorbance at 220 nm using a spectrophotometer, without the use of a nitrate test kit. To use this method to accurately measure nitrate concentrations. It works well for solutions containing only a nitrate compound, but unable to find a simple method by which we could

accurately determine the nitrate concentrations in our (filtered) NRP samples. The other compounds in the samples seem to inhibit accurately measuring the nitrate concentrations. The procedure outlined in the *Ultraviolet Spectrophotometric Screening Method* instructs the experimenter to subtract twice the absorbance at 275 nm from the absorbance at 220 nm. This method proved quite inadequate for samples. The absorbance at 275 nm was at times very small and yielded a meaningless correction. At other times, the absorbance at 220 nm was much less than it should have been, so any additional correction would have made the measured concentration even more inaccurate.

From Figure 8, the measured nitrate concentrations are significantly higher when using the test kit. The concentrations without the test kit follow the same increasing-decreasing pattern (which corresponds to the nitrification-denitrification process), which is encouraging. This means that the observed absorbance values likely correspond somehow to the nitrate concentration [23,24]. Nonetheless, we have been unable to determine an appropriate correction factor that can be used to turn these absorbance values into actual nitrate nitrogen concentrations.

4. Conclusions

It was concluded that experiments have led us to the conclusion that it is more difficult to achieve nitrogen removal within the reactor than it is to achieve removal of BOD. A longer residence time seems to be necessary to allow for complete nitrification and denitrification. Aerating the reactor at high DO levels, will likely remove all BOD long before the process of nitrification has continued to completion. Denitrification also seems to be a slow process. Further experimentation is necessary in order to compare the efficiency of denitrification when using various electron donors. It is likely that a process that utilizes the synthetic waste as electron donor (e.g. the Barnard Process) would allow for more efficient denitrification. It seems to be quite difficult to coax your reactor into the nitrification-denitrification process if you have a short residence time. It would be more feasible to perform an experiment (say, comparing the modified Barnard Process described above to the biomass storage and decay process that we employed) if shoot for a residence time of 12 hours or more. This will probably allow you to select for bacteria effective at nitrogen removal early on in the project, and will give you more time to do experimentation.

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