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Nutrient Recycling from Aqueous for Nitrogen Supplementation in Algae Growth

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ABSTRACT

Algae-derived biofuels have the potential to become a source of renewable liquid fuel via hydrothermal liquefaction. However, for algal biofuels to be economically and environmentally feasible, sustainable nutrient recycling must be achieved. *Desmodesmus armatus* is a microalga to be used in hydrothermal liquefaction, but it is not yet known if the aqueous product waste from the biofuel production process can be recycled as a nitrogen source to support the growth of subsequent cultures of *D. armatus*. Here, aqueous product was treated with a Dowex 50WX8 resin for 24 hrs. Growth media was prepared with treated and untreated aqueous product at 25% nitrogen supplementation for culturing *D. armatus*, and growth was tracked using optical density measurements. We found that growth rates between the untreated, treated, and control conditions were similar, and not significantly different. This indicates that the recycling of aqueous product from hydrothermal liquefaction for the growth of *D. armatus* is a sustainable way to achieve nutrient recycling for algae biofuels. If it is possible to recycle aqueous product directly from hydrothermal liquefaction to growth media, then the overall process of algae biofuels will become more economically feasible than using the treated aqueous product. This study demonstrates, for the first time, the possibility of using untreated aqueous product for use in growing *D. armatus*.

Keywords: Aqueous product, nutrient recycling, algae biofuels, biofuels, hydrothermal liquefaction

INTRODUCTION

As the world's energy demand continues to increase, the production of renewable forms of energy will become even more important to the global energy portfolio. In the last five decades, there have been many advancements in renewable energy, including the creation of fuel from microalgae via hydrothermal liquefaction (HTL) (Jiang et al., 2019; Laurens, 2017; Leng et al., 2018). Microalgae biofuels have the potential to replace traditional nonrenewable sources of liquid fuels. Algae are uniquely suited to become a feedstock -or source- for biofuel production because of their ability to be cultivated at mass scale, as they are fast-growing and can be grown on non-arable lands (Elliott et al., 2015). The microalgae species *Desmodesmus armatus* (*D. armatus*) is a particularly promising microalgae species. *D. armatus* is known for its ability to grow in non-ideal conditions and its high lipid content (P. H. Chen et al., 2020).

HTL of microalgal biomass yields a liquid fuel that is comparable to current liquid gasoline, allowing for the possibility of direct supplementation of liquid fossil fuels with little to no modification of current technologies. HTL is the application of high temperature and pressure to a biomass slurry to create mainly a bio-crude oil (Elliott et al., 2015; Juneja et al., 2013; Laurens, 2017; Shakya et al., 2017). There are three products produced from HTL; biocrude oil, aqueous product (AP), and char (Leng et al., 2018; Shakya et al., 2017; Vo et al., 2017). A general process flow diagram can be seen below (Figure 1).

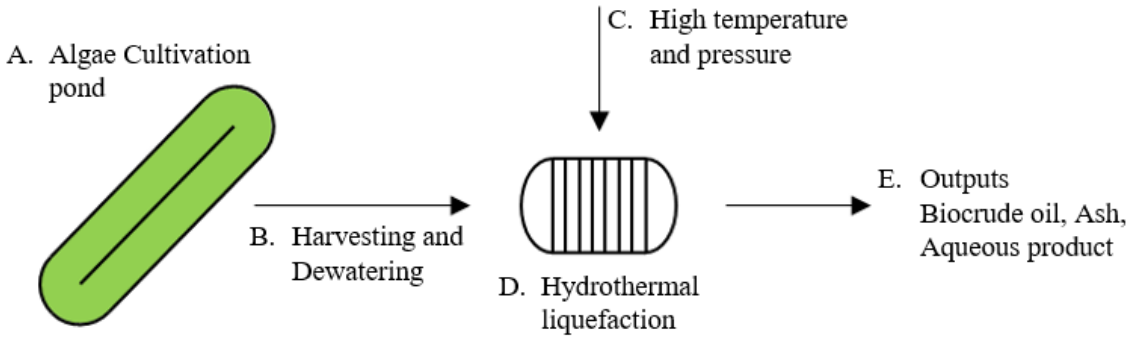

Figure 1*:* General process flow diagram for a hydrothermal liquefaction process.
General process flow diagram for a hydrothermal liquefaction process showing A) The cultivation of microalgae B) the harvesting a dewatering of the microalgae C) The application of high temperature and pressure via D) hydrothermal liquefaction E) biocrude oil, ash, and aqueous product.

HTL is a promising fuel conversion method because it does not require a feedstock that has gone through a costly drying processes (Quinn & Davis, 2015). However, bio-crude oil from the HTL of microalgae tends to contain high levels of nitrogen and oxygen, meaning further upgrading is required to meet national fuel standards, which require low levels of NO_x emissions (Wang & Tao, 2016). This increases the cost of HTL derived biofuels. Another aspect leading the increased costs of HTL-derived biofuels is the inconsistency in the output from HTL. HTL products are highly dependent upon input feed, changing based upon biomass composition, wt.% biomass, and media used to grow the biomass. Due to the multiple input variables, it is difficult to predict and classify the outputs of HTL leading to increased costs.

Currently, microalgae biofuels from HTL are predicted to cost $4.35–$4.49 per gasoline gallon equivalent (GGE) (Davis et al., 2016). The United States Department of Energy Bioenergy Technologies Office has set a goal of $3/GGE for liquid biofuels. Therefore, the cost of HTL-derived biofuels from microalgae must decrease significantly to reach this goal (Davis et al., 2016).

One way to potentially decrease the costs of microalgae biofuels is through nutrient recycling. Growing microalgae requires substantial amounts of the nutrients phosphorus and nitrogen (Juneau et al., 2013). The demand for nutrients makes microalgae biofuels currently unfeasible, as the creation and acquisition of fresh nutrients can be detrimental to the environment and cost prohibitive (Davis et al., 2016; Laurens, 2017). Estimates done by Davis et al. suggest that 22% of the minimum biomass selling point is accounted for by CO_2 and nutrient inputs for an average 10-acre pond (Davis et al., 2016). If nutrients can be recycled back into the growth media used for growing microalgae, then fewer fresh nutrients will need to be used, and the cost of producing microalgae biofuels can be decreased. Nitrogen is one of the main nutrients needed for microalgae growth, and previous work has shown that the nitrogen content in microalgae growth media may be supplemented with nitrogen from a waste product of HTL (P. H. Chen et al., 2020).

HTL yields two main waste products: aqueous product (AP) and char (Leng et al., 2018; Shakya et al., 2017; Vo et al., 2017). AP has been shown to retain a high percentage of the nitrogen found in the feedstock used for conversion (Shakya et al., 2017). Moreover, microalgae will grow in AP that has been diluted into other growth mediums (Biller et al., 2012; Garcia Alba et al., 2013). A

study done by Biller et al. tested 5 different microalgae species and found that all had inhibited growth at 50x dilution of AP, but growth was improved once 200x dilution of AP was achieved. A similar study by Alba et al. showed that the microalgae species *Desmodesmus sp.* can grow in solutions containing demineralized water, standardized growth media and AP at approximately 50, 49.75, and .25 wt.% respectively. There was no significant difference between growth rates for *Desmodesmus sp*. grown in the given solution and those that grew in standard growth media. The researchers also found that there are compounds in AP that can inhibit microalgae growth such as toxic organic compounds or ammoniacal nitrogen (Garcia Alba et al., 2013).

Because of the hypothesis that there are toxic components in AP, multiple studies treating AP with different adsorbents have been attempted. These studies have cited improved growth of microalgae in media containing AP treated with various adsorbents when compared to growth in media containing untreated AP. These studies used adsorbents such as cation exchange resins and granulated active carbon (K. Chen et al., 2015; P. H. Chen et al., 2020; Fushimi et al., 2016). However, these studies have not definitively shown that the compounds removed by the adsorbents inhibit growth of microalgae, and there has been little work done to classify which compounds these adsorbents removed from the AP. As a result, the inhibited growth could be caused by something other than compounds in the AP.

One adsorbing agent that shows promise is a cation exchange resin called Dowex. This resin has been shown to improve the growth of *Chlorella sp.* when growth in treated AP is compared to growth in untreated AP (P. H. Chen et al., 2020). In 2020, P.H. Chen reported that there was a linear growth rate of approximately 0.3 grams per day for *Chlorella sp.* grown in both traditional growth media and media containing AP treated by Dowex and diluted to 100x. This is an improvement over a linear growth rate of approximately 0.1 grams per day for *Chlorella sp.* grown in traditional growth media containing untreated AP diluted to 100x (P. H. Chen et al., 2020).

D. armatus is a microalgae species that has some promising properties for HTL due to its robust growth in outdoor conditions and attractive biomass composition for conversion (P. H. Chen et al., 2020). These properties are due to *D. armatus'* ability to grow in adverse conditions such as non-optimized media and in the presence of toxins. There is a large interest in testing *D. armatus'* response to growth in AP. However, there is little known about the AP derived from *D. armatus* and how Dowex treatment would affect subsequent growth.

In this study, we investigated how AP derived from the HTL of *D. armatus* would affect the growth of *D. armatus*, and the ability for treatment with Dowex 50WX8 to improve growth. Growth trials were performed using *D. armatus* to determine the feasibility of nitrogen recycling from AP for microalgae growth. We hypothesized that media containing treated AP from the HTL of *D. armatus* would lead to improved growth rates when compared to growth media containing untreated AP.

MATERIALS AND METHODS

HTL aqueous phase

D. armatus was harvested from two 75 L raceway ponds. Once harvested the algae was dewatered via centrifugation until the dry weight was between 20-30%. This created an algal slurry that was

used for HTL. Four 75 mL Parr reactors were loaded with approximately 25g of algae slurry each. The reaction vessels were purged prior to heating, and HTL was performed at 300°C for 30 min. After the reaction was complete, the AP was separated from the biocrude through gravity filtration. The AP was then passed through a 0.22-micron filter to remove the remaining ash.

Aqueous product treatment

The isolated AP (40 mL) was placed in a beaker with 8g of Dowex 50WX8 resin, and the mixture was covered and placed on a stir plate for 24 hrs. The treated AP was separated from the spent Dowex resin using a Buchner funnel with .22-micron filter paper. The AP was filtered three times to ensure full removal of Dowex resin.

Adsorbate removal

The Dowex resin used to treat the AP was washed with various solutions to remove some of the adsorbates. The washes used were dichloromethane: methanol (50:50 v/v) with acetic acid (10%); methanol: acetic acid (95:5); water: acetic acid (95:5). All washes were performed 3 times, with separation of the Dowex resin from the wash via vacuum filtration performed after each rinse. The washes were then combined and dried under nitrogen. The removed adsorbates were re-dissolved using water: 95% ethyl alcohol (90:10). This method was also applied to *Chlorella vulgaris (C. vulgaris)* for comparison with previous work. The removed adsorbates from *D. armatus* that were re-dissolved were used to create growth media.

Mass balance

Mass balance calculations were performed to determine the mass of material removed from the Dowex resin. Three 1mL samples from the untreated AP and the treated AP were taken and dried under nitrogen. Three 1mL samples from the three Dowex washes were also dried under nitrogen. After evaporation under nitrogen, the samples were weighed to determine the mass yield of each wash in comparison to the treated and untreated AP samples.

Nitrogen determination

The amounts of carbon, hydrogen, and nitrogen present in untreated AP, treated AP, and the adsorbates removed from *D. armatus* were analyzed by carbon, hydrogen, nitrogen combustion analysis (Table 1). The information gathered was used to calculate the amount of each solution needed to reach 2.5 mM nitrogen (Table 1). All growth trails contained 10 mM nitrogen per liter of growth media, hence 2.5 mM of nitrogen represents 25% nitrogen replacement for one liter of growth media.

TABLE 1: NITROGEN PRESENT IN VARIOUS SOLUTIONS

Amount of solution needed for 2.5 mM nitrogen in 1 L of growth media determined by CHN combustion analysis.

Solution	**Nitrogen % in solution**	**Amount of solution needed for 2.5 mM of nitrogen in 1 L of media**
Untreated AP	0.67	5.05 mL
Treated AP	0.23	15.78 mL
Adsorbate solution	0.12	30.75 mL

Algae cultivation and analysis

Different conditions were created and tested to demonstrate how *D. armatus* grows in AP. All media contained modified artificial sea water media (MASM) without any nitrogen sources added (Table 2). Once created, all media were adjusted to a total nitrogen concentration of 10 mM using NH_4Cl and recycled HTL aqueous phase. The following media were created, and growth trials for each media were performed in triplicate (Figure 2).

TABLE 2: RECIPE FOR MASM USED AS BASE MEDIA IN GROWTH TRIALS

Component	**Amount per Liter**
NaCl[1]	8.0 g
$MgSO_4 \bullet 7H_2O$	2.49 g
KCl	0.6 g
$CaCl_2 \bullet 2H_2O$	0.3 g
Tris base	1.0 g
$NaHCO_3$	0.84 g
KH_2PO_4	2 ml of a 25 g/l mixture
CM T.E.	6.0 ml
Thiamine and B_{12} mixture	0.5 ml

[1] The repeated growth trial did not contain any NaCl

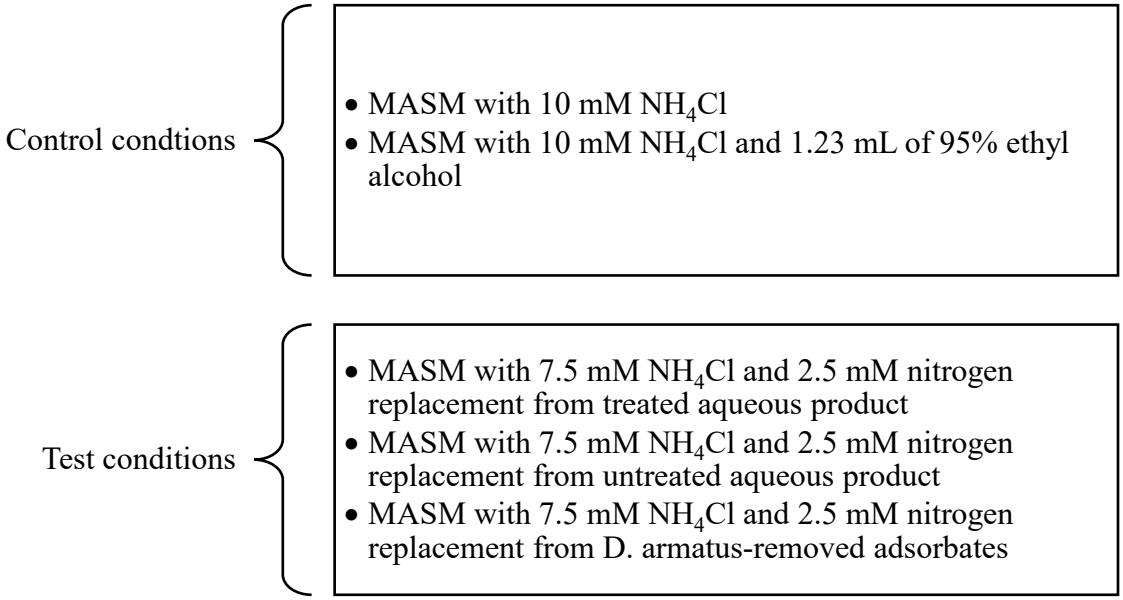

Figure 2: Summary of growth conditions.
Summary of growth conditions used to test the effects that untreated and treated AP has on the growth of microalgae species *D. armatus*. Growth conditions all contained 10 mM total nitrogen.

Since the solution containing the adsorbates removed from *D. armatus* contains 95% ethyl alcohol, there was a possibility that the ethyl alcohol would inhibit growth. To control for this, a media was created with 10 mM NH_4Cl and 1.23 mL of 95% ethyl alcohol. This control media contains the same amount of ethyl alcohol that is in the experimental condition for the removed adsorbates.

Growth media were inoculated with wild type *D. armatus* to an optical density (OD) at 750 nm (OD_{750}) of approximately 0.1. All conditions were repeated in triplicate with a working volume of 125 mL in 500mL flasks with foam tops. All flasks were placed on shaker plates with consistent light of 50-60 μm with temperatures ranging from 24-26ºC. Optical density measurements, at 750 nm, were taken every weekday to develop growth curves for each experimental condition. All trials were compared with a blank of a sample of the growth media for that experimental condition.

A repetition of the experiment was performed to increase confidence in the results. The setup for the second replicate was slightly altered due to space and material resource availability. All conditions for the second replicate were performed in triplicate with a working volume of 50 mL in 250 mL flasks with foam tops. MASM without NaCl or nitrogen was used as the base media. Light was supplied consistently at 30 μm, and the temperature was constant (24ºC). Because different light conditions and timing were used, the two trials were examined separately.

Contamination
To monitor for the interference of bacterial growth on reported data, appropriate measures were taken to determine when or if a growth trial was dominated by bacterial growth rather than algae growth. These measures included OD taken at 680 nm, visual inspection, and microscopy.

Growth curves at 680 nm

Growth curves for both the initial and repeated growth trials were developed for a wavelength of 680 nm in addition to the growth curves developed at 750 nm. If the growth curves between the 750 nm and 680 nm wavelengths showed similar trends, then it was determined that the condition was not overly contaminated. Growth curves at 680 nm can be found in Appendix B.

Visual inspection

Each day, flasks were visibly inspected to determine the degree of contamination. If the growth conditions were good (*i.e.,* small amounts of bacteria growth), the samples appeared a dark green color when mixed and had longer settling times. After settling, a clear media appeared on top. Experimental conditions that became more contaminated appeared a pale green when mixed and settled more quickly than the other conditions. After settling, a cloudy media appeared on top. Images showing the characterization of the different trials can be found in Appendix C

Microscopy

Aliquots from each sample were observed under a microscope at 40x magnification to determine the condition of each flask. If a culture had a high density of algae with little to no background bacteria, it was considered usable. Experimental conditions with a high density of bacteria and lots of debris were considered overly contaminated and not used. Only cultures that had both a high density of bacteria and high mobility were categorized as contaminated. Example images from microscopy with descriptions can be found in Appendix D.

RESULTS

Mass balance

Mass balance calculations were performed to quantify the effects of treating AP with a Dowex 50WX8 resin. Untreated and treated AP were dried down under nitrogen to determine the mass present in 1 mL of respective AP samples. Percent recovery was then determined by comparing the mass found in 1 mL of untreated AP to the mass found in 1 mL of treated AP. To determine the percent recovery of material from washing the spent Dowex resin, the same procedure was performed on the three different solutions that were used to for washing the Dowex. A summary of these results can be found in figure 3.

The development of the solutions used to wash the Dowex resin used to treat the *D. armatus* AP was developed using AP from the microalgae species *C. vulgaris*. Dowex 50WX8 resin was used to treat AP from the HTL of *C. vulgaris.* The spent Dowex resin was washed with various solutions until the optimum method for removing material was found. The following three solutions were found to be the most effective: a) dichloromethane: methanol: acetic acid, b) methanol: acetic acid, c) water: acetic acid. Mass balance calculations were performed as described to determine the percent recovery of mass in the treated AP and removed adsorbates for *C. vulgaris* and *D. armatus.* The results are shown in figure 3.

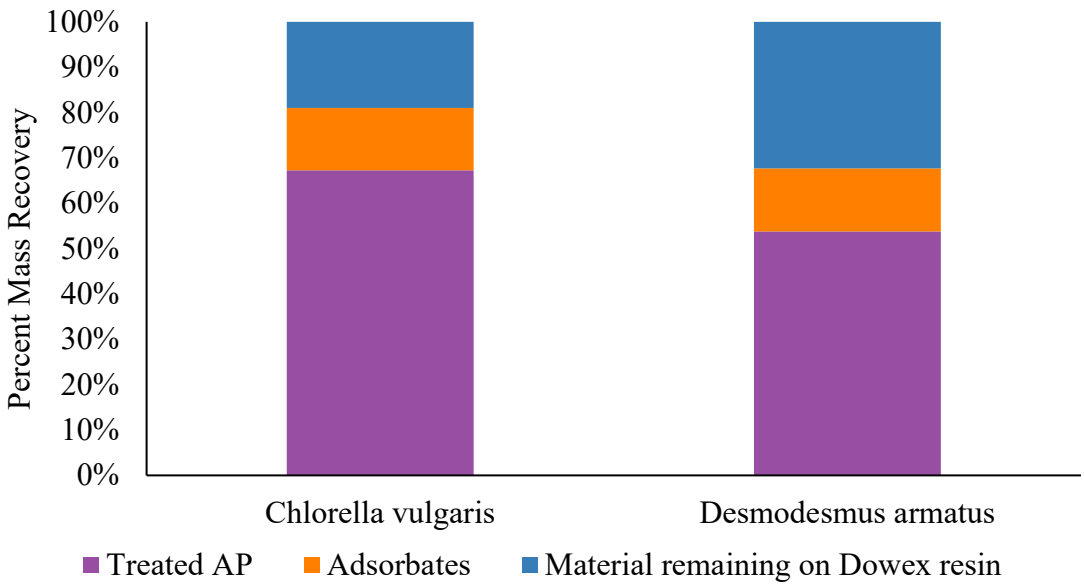

Figure 3: Comparison of percent mass recovery from *C. Vulgaris* and *D. armatus.*
When looking at 1 mL samples of the following solutions dried under nitrogen in comparison to untreated aqueous product from the two microalgae species. Solution one – aqueous product from *C. vulgaris* and *D. armatus* treated with a Dowex 50WX8 resin. Solution two – adsorbates removed from spent Dowex resin used the following washes: a) dichloromethane: methanol: acetic acid, b) methanol: acetic acid, c) water: acetic acid.

C. vulgaris had a 67% recovery of the treated AP fraction compared to 53% for *D. armatus*. The adsorbates exhibited a percent recovery of approximately 13% in both cases. The remaining fraction is the material that was left of the Dowex 50WX8 resin. Comparison of these samples also reveals that *D. armatus* resulted in a greater quantity of irreversibly adsorbed compounds when compared to *C. vulgaris* (34% vs. 20%). This is likely due to greater molecular polarity in the HTL aqueous phase for *D. armatus* which makes recovery from the resin more difficult. Further work is necessary to determine if these highly polar compounds may be removed and what chemical properties lead to their strong interaction with Dowex.

Algae cultivation and analysis

Figure 4 shows the growth curves for the first trial of the experiment. The growth curve was developed by taking ODs at 750 nm. The treated AP, untreated AP, and control conditions all exhibited comparable growth rates throughout the experiment. We had hypothesized that the untreated AP would yield the lowest growth rate and that the treated AP would yield the highest growth rate. This is not supported by the data, since the growth rates between the untreated AP and treated AP were comparable.

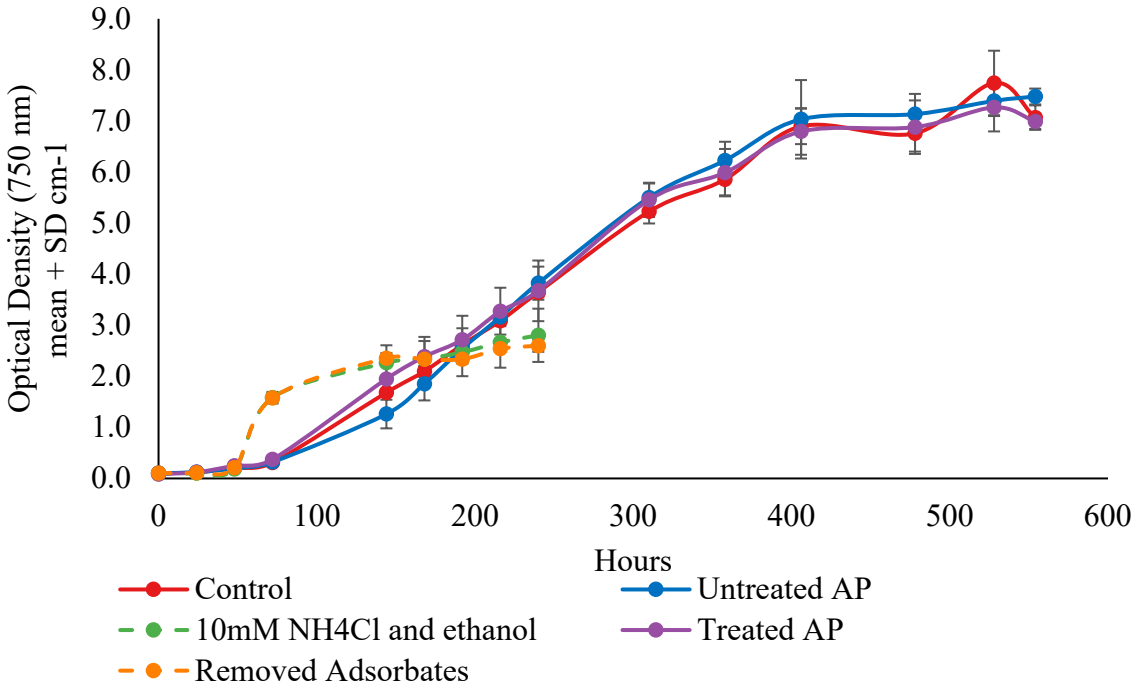

Figure 4: Growth curves for second growth trial with OD taken at 750 nm.
Growth conditions were as follows: control conditions: MASM with 10 mM NH_4Cl, MASM with 10 mM NH_4Cl and 1.23 mL of 95% ethyl alcohol. Experimental conditions: MASM with 7.5 mM NH_4Cl and 2.5 mM nitrogen replacement from treated aqueous product, MASM with 7.5 mM NH_4Cl and 2.5 mM nitrogen replacement from untreated aqueous product, MASM with 7.5 mM NH_4Cl and 2.5 mM nitrogen replacement from adsorbates removed from *D. armatus*. Growth trials were grown in triplicate.

The first trial showed similar results, and growth curves for the first growth trial can be found in appendix A. The treated AP conditions, untreated AP conditions, and control conditions all exhibited comparable growth rates. For both experiments, the 10 mM NH_4Cl- ethanol and removed adsorbate trials became overly contaminated with bacteria early in the growth period, yielding inconclusive data.

DISCUSSION

This study sought to characterize the effects of nitrogen supplementation with treated or untreated AP on microalgae growth. We found that both treated and untreated AP lead to comparable growth rates. In addition, these growth rates were equivalent with the growth rates from the control trials using ammonium chloride, indicating that both treated and untreated AP can be used in nitrogen supplementation for the growth of *D. armatus.* This is significant because other species of algae have not shown the ability to grow in untreated AP. If untreated AP can be used for nitrogen supplementation in growth media, the overall process of algae biofuel production will become more economically feasible and environmentally friendly.

The data gathered from this experiment indicate that the growth of *D. armatus* is not affected by either untreated or treated AP when 25% nitrogen replacement is used as a basis. The optical density for the control, untreated, and treated AP samples were within the range of 7-7.5 absorbance units (once corrected for dilution) during the repeated growth trials. In addition, none

of these trials showed high levels of contamination, indicating that the *D. armatus* microalgae cells were not excreting extracellular polymeric substances. Therefore, they were not stressed. As the *D. armatus* cultures were not stressed by the introduction of treated or untreated AP, it would be beneficial to determine the extent to which nitrogen supplementation via AP can be achieved. A study done by P.H. Chen et al. examined the relationship between microalgae growth and varied percent nitrogen supplementation from Dowex 50WX8-treated AP. This study examined the growth of *C. vulgaris*. The researcher found that *C. vulgaris* growth rate, with nitrogen supplementation from Dowex-treated AP (to a level of 35% nitrogen replacement), were comparable to control condition growth rates. A similar methodology could be used to determine the extent to which nitrogen supplementation from untreated AP can be performed for *D. armatus.*

The conclusion that *D. armatus* yields comparable growth rates for treated AP and control condition is in line with the current literature. P.H. Chen et al. determined that *C. vulgaris* has comparable growth rates for treated AP and control conditions when a Dowex resin is used to treat the AP (P. H. Chen et al., 2020). Multiple studies cite improved growth rates of microalgae in treated AP over untreated AP, indicating that untreated AP contains compounds that inhibit the growth of microalgae (Biller et al., 2012; P. H. Chen et al., 2020; Garcia Alba et al., 2013). *D. armatus* did not show inhibited growth rates in media containing untreated AP at 25% nitrogen replacement (approximately 5 wt.%), showing that either AP from the HTL of *D. armatus* does not contain growth-inhibiting compounds or that *D. armatus* is robust enough not to be affected by any growth-inhibiting compounds, that may be present in the untreated AP. To determine whether the AP from the HTL of *D. armatus* contains growth-inhibiting compounds, it may be beneficial to test the growth of other algae species in untreated AP from the HTL of *D. armatus.* It may also be beneficial to test algae growth by increasing the percent nitrogen replacement in the growth media.

The uninhibited growth of *D. armatus* when in the untreated AP media was unexpected. All other studies indicate that microalgae do not grow as well in untreated AP (K. Chen et al., 2015; P. H. Chen et al., 2020; Fushimi et al., 2016). For example, a study done by P.H. Chen showed that *Chlorella sp.* had comparable growth rates when grown in traditional growth media and when grown in traditional growth media with Dowex-treated AP. The rates were approximately 0.3 grams per day when the treated AP was diluted 1:100. *Chlorella sp.* grown in traditional growth media with untreated AP had a linear growth rate of approximately 0.1 grams per day (P. H. Chen et al., 2020). However, this is the first study performed on *D. armatus* growth in media containing AP. This is a novel finding that will have to be investigated farther.

It is important to note that in both iterations of the growth trials, the conditions containing ethanol (10 mM NH_4Cl and ethanol, removed adsorbates) experienced unexpected bacterial growth – indicating that additional experimental controls should be used in any repetitions of this experiment. Since both trials included the addition of ethanol, it is possible that the presence of ethanol is responsible for the increased bacterial growth. This finding is not supported by the literature, but no studies have been conducted exploring the relationship between algae growth and ethanol. The exact reason why the two trials containing ethanol showed high levels of bacterial growth is unknown. One reason is that ethanol could have caused bacterial growth by stressing the *D. armatus* cultures, leading to the excretion of exopolysaccharides. Bacteria can feed off exopolysaccharides, which may have led to the high level of bacterial growth for these two trials.

Future work investigating a possible link between ethanol and bacterial growth or ethanol and inhibited algal growth of *D. armatus* may prove insightful.

If untreated AP can be used in nitrogen supplementation for the growth of *D. armatus*, the overall process of algae biofuel production will become more economically feasible and environmentally friendly. It is difficult to quantify the effects of nitrogen supplementation because there is not enough information available about *D. armatus* as a feedstock for algae biofuels. It can be assumed, however, that if nitrogen can be recycled without treatment, fewer fresh nutrients would be required; and the cost of algae biofuels would decrease. However, the extent to which this may affect the cost of biofuels from *D. armatus* is not known. It can also be assumed that if nitrogen no longer needs to be produced at mass scale to grow algae biofuels, then it becomes a more environmentally friendly operation.

This study showed that *D. armatus* can grow in media containing 25% nitrogen replacement from untreated AP. More work should be done to determine the extent to which nitrogen can be replaced by either treated or untreated AP. In addition, work should be done to determine the chemical composition of AP derived from HTL of *D. armatus* for comparison with AP derived from other microalgae species. This may give a better understanding of why *D. armatus* was able to grow in untreated AP when other algae species have not. The development of commercial scale algae biofuels operations appears to be on the horizon.

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APPENDIX A

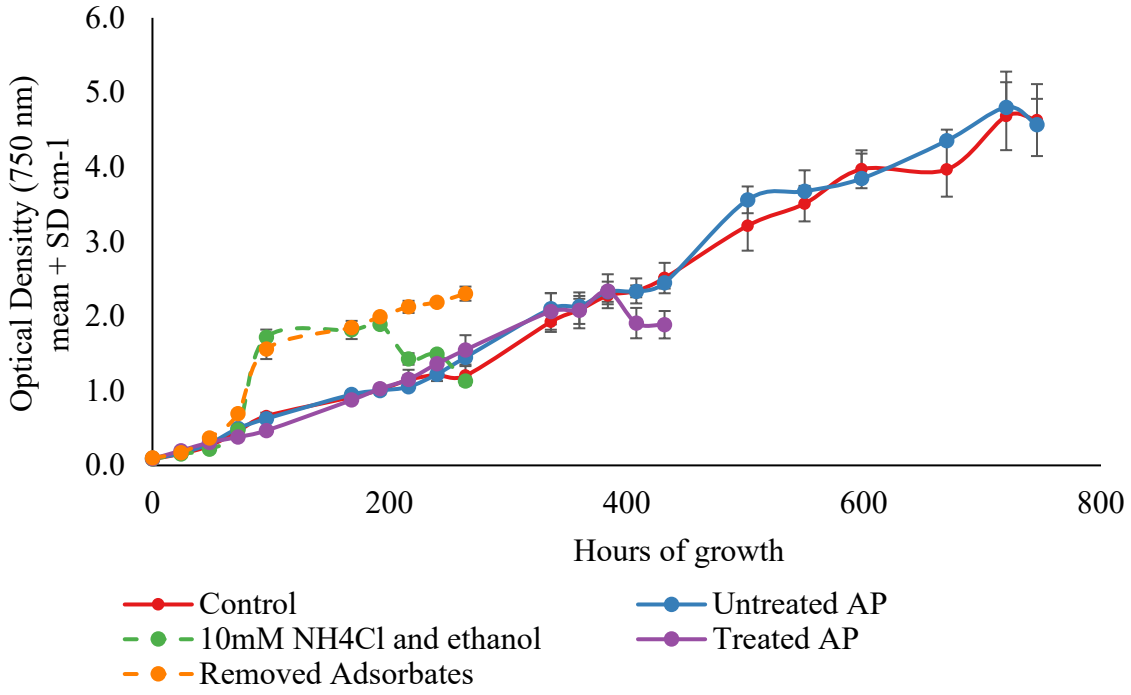

Figure A1: Growth curves for initial growth trial with OD taken at 750 nm.
Growth conditions were as follows. Control conditions: MASM with 10 mM NH_4Cl, MASM with 10 mM NH_4Cl and 1.23 mL of 95% ethyl alcohol. Experimental conditions: MASM with 7.5 mM NH_4Cl and 2.5 mM nitrogen replacement from treated aqueous product, MASM with 7.5 mM NH_4Cl and 2.5 mM nitrogen replacement from untreated aqueous product, MASM with 7.5 mM NH_4Cl and 2.5 mM nitrogen replacement from adsorbates removed from *D. armatus*. Growth trials were grown in triplicate. Dashed lines indicate contaminated cultures.

APPENDIX B

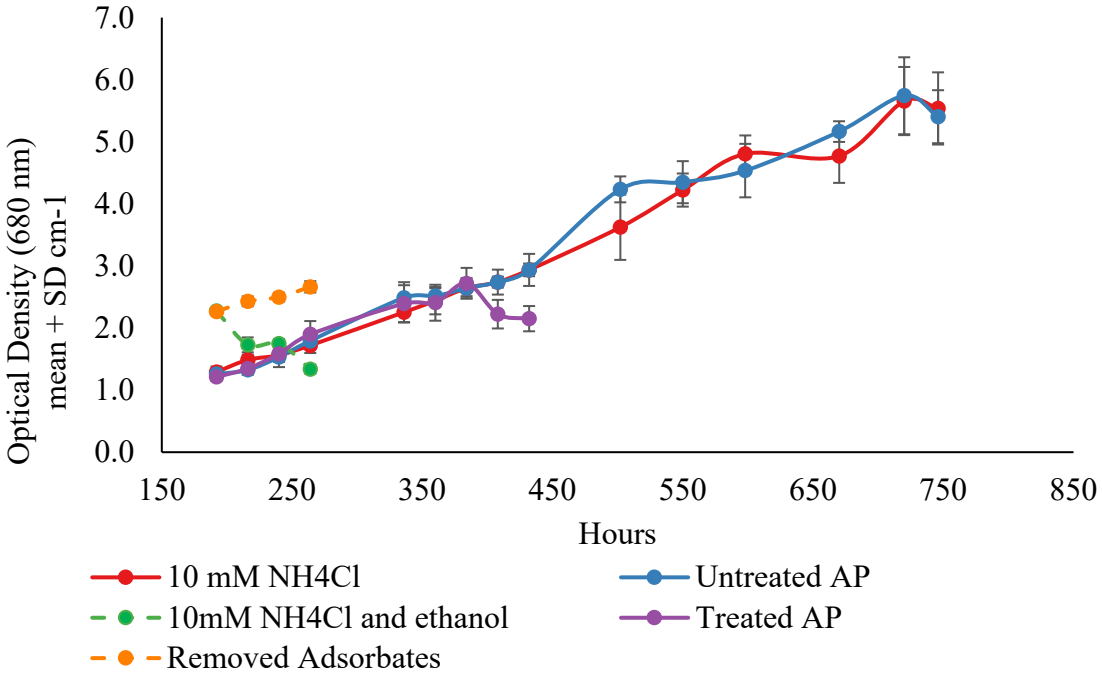

Figure B1: Initial growth curve of *D. armatus* at 680 nm.
Growth conditions were carried out as follows. Control conditions: MASM with 10 mM NH_4Cl, MASM with 10 mM NH_4Cl and 1.23 mL of 95% ethyl alcohol. Experimental conditions: MASM with 7.5 mM NH_4Cl and 2.5 mM nitrogen replacement from treated aqueous product, MASM with 7.5 mM NH_4Cl and 2.5 mM nitrogen replacement from untreated aqueous product, MASM with 7.5 mM NH_4Cl and 2.5 mM nitrogen replacement adsorbates removed from *D. armatus*. Growth trials were grown in triplicate. Dashed lines indicate contaminated cultures.

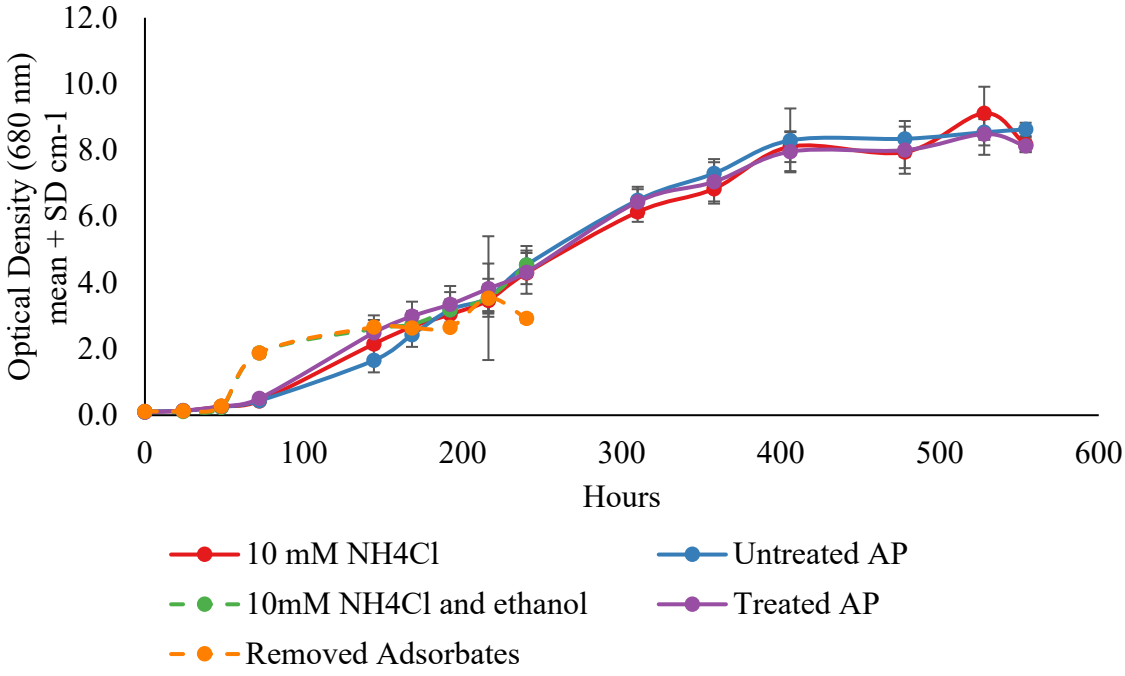

Figure B2: Repeated growth cure of *D. armatus* at 680 nm.
Growth conditions were as follows. Control conditions: MASM with 10 mM NH_4Cl, MASM with 10 mM NH_4Cl and 1.23 mL of 95% ethyl alcohol. Experimental conditions: MASM with 7.5 mM NH_4Cl and 2.5 mM nitrogen replacement from treated aqueous product, MASM with 7.5 mM NH_4Cl and 2.5 mM nitrogen replacement from untreated aqueous product, MASM with 7.5 mM NH_4Cl and 2.5 mM nitrogen replacement adsorbates removed from *D. armatus*. Growth trials were grown in triplicate. Dashed lines indicate contaminated cultures.

APPENDIX C

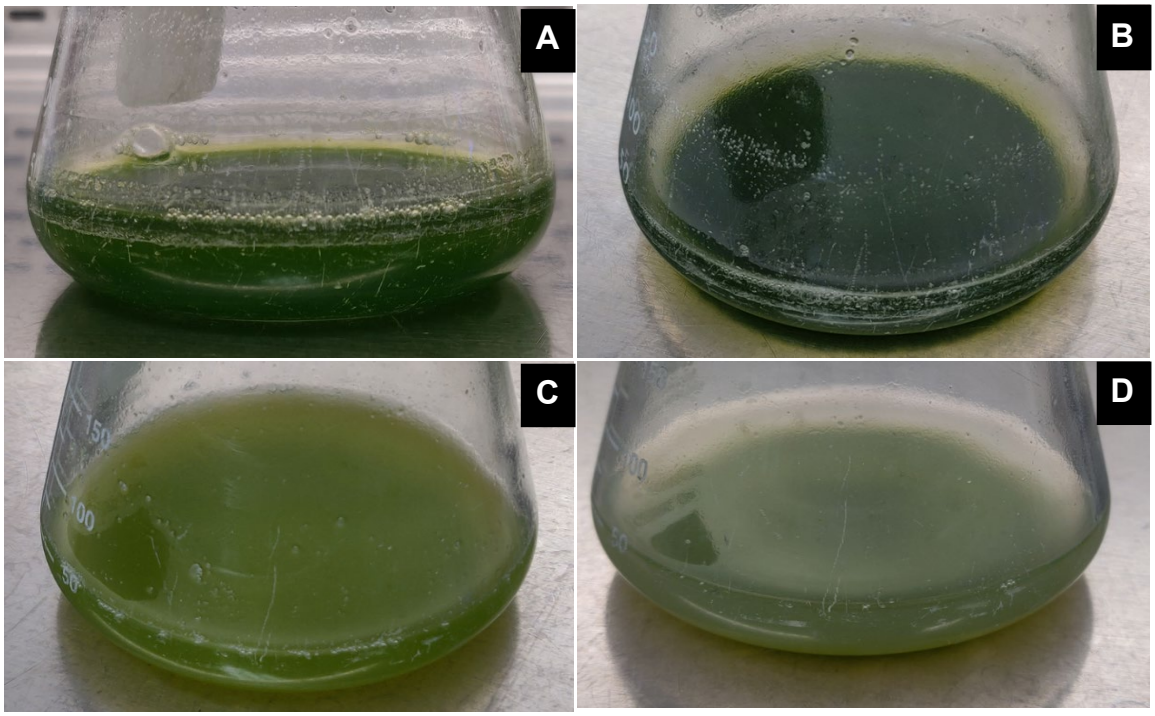

Figure C1: Images for visual inspection of various conditions.
Examples of cultures; (a) Control condition agitated culture exhibiting a dark green uniform color indicating minimal contamination (b) Control condition settled culture with clear media on top layer indicating minimal contamination. (c) 10 mM NH_4Cl and ethanol agitated culture exhibiting light green inconsistent coloring indicating contamination. (d) 10 mM NH_4Cl and ethanol settled culture with opaque media on top layer indicating contamination.

APPENDIX D
MICROSCOPY INSPECTION OF VARIOUS CONDITIONS

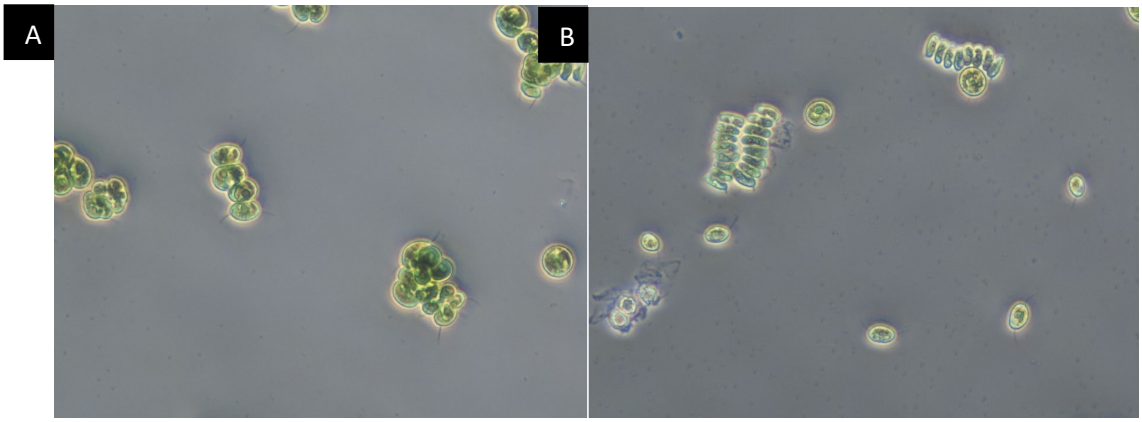

Figure D1: Microscopy images of a cultures that were not contaminated.
A)Image A shows a control condition culture expressing a high density of *D. armatus* cells with little background bacteria. B) Image B shows a treated AP culture expressing a higher density of bacteria with no mobility, culture still possessed a high density of *D. armatus* cells.

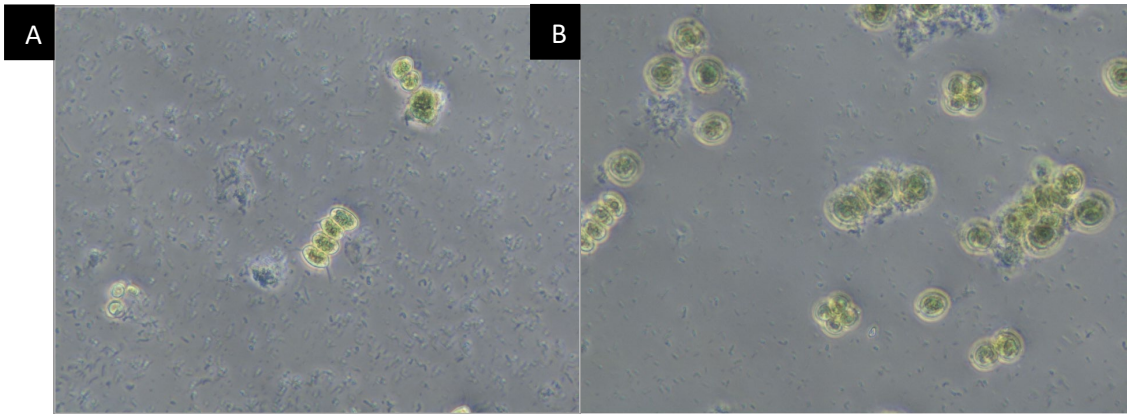

Figure D2: Microscopy images of cultures that were contaminated.
A) Image A shows a removed adsorbate culture expressing a high density of bacteria and debris, bacteria had high motility, density of *D. armatus* cells was low. B) Image B shows a 10 mM NH_4Cl and ethanol culture expressing a moderate density of bacteria with high mobility, culture still possessed a moderate density of *D. armatus* cells.