

Please find the peer reviews and responses for this article submitted to PeerJ for publication below:

1. The abstract is given twice and is far too long. It should be cut down by at least half.

The abstract has been cut down substantially and redundancy has been removed. It should now only appear once in the manuscript.

2. There are many unsubstantiated and often false statements.

A. In what sense were attempts to create fuel from fats and oil unsuccessful? Making fuel from these sources is easily accomplished and there is an industry that does this. In any case statements like this need a reference.

Lines 37-38 have been added with a reference to answer this question.

B. It is unclear what you mean by second, third and fourth generation biofuel since according to the author, they are all algae-derived.

Lines 40-44 have been added to explain the difference between second, third, and fourth generation biofuel.

3. The term individual lipid production per set of biomass is ambiguous. These terms are ambiguous and not in accord with normal usage. What is the individual here? What is the set? The normal measure of biomass is dry weight.

I corrected the usage in line 62- it is now “cellular lipid production per dry weight of biomass.”

4. line 62 The growth of algae is certainly important in determining costs but so is processing (harvesting, extraction and conversion to biodiesel).

Lines 70-71 now includes this information.

5. line 63 Quality? It should be lipid content.

This has been corrected.

6. Line 70 Needs a reference. There is a reference here to something previously published but the reference is not given.

The reference is now added (line 73).

7. Line 73 Whose initial experimentation?

A reference has been added on line 77 to show who performed the initial experimentation.

8 Line 84 “The experiment has a total of four treatment levels” This is incorrect. There are only two treatments (with or without NP) that are applied to two different species.

This has been fixed to include only two treatment levels (line 87).

9. Line 87. The reference needs to be cited properly.

It is now cited properly (line 91).

10. Line 99 You have a third variable here (Potassium) that also differs from the “controls” but which you ignore.

I understand your concern. The manuscript has been updated to reflect potassium as a variable.

11. Line 117 Lipids are readily trapped in with the particulates. Is there any way to know what % of the lipids were filtered out with the particulates.

Unfortunately, there is no way to know what % of the lipids were filtered out with the particulates. I have included in the manuscript now the size of the particulates that were filtered (line 125) which may assist in providing additional information to the reader regarding this topic.

12. While hexane will selectively dissolve triacylglycerols, it is not very efficient at extracting lipids from an aqueous solution because it does not mix with water. Furthermore, there are lipid like compounds such as carotene that will readily dissolve in hexane.

Hexane is commonly utilized in lipid extractions for microalgal strains. Hexane is easier to separate from the lipids after extraction than other compounds such as toluene. This is

because hexane is easier to evaporate and thus was used in this study. Furthermore, this study does not attempt to extract lipid-like compounds such as carotene.

13 The rationale for using “salt” to lyse cells is not given. In the methods you said that the algae were lysed by heating. You gave no reason for why you add salt nor any justification. Furthermore, you did not explain why the two algae were treated differently. Salt being ionic, makes it even more difficult to mix hexane (very hydrophobic) with water and thus to extract lipids.

Because the *Nannochloropsis sp.* is a salt water strain, it was necessary to add more salt than the freshwater strain, in order to degrade the cell membranes/wall for lipid extraction as this strain is acclimated to being in high salt conditions. I swirled the hexane mixture very thoroughly in order to extract as many lipids as possible.

Note:

The line numbers shown above correspond to the manuscript draft.