

Assalamualaikum wbh,

Dear Dr Anis Nurasyikin Nordin,

These are the comments from reviewer B and the answer for each comment.

1. The paper claims to estimate the protein content in earth worms using Kjeldahl method. However, Kjeldahl method provides nitrogen content from all the nitrogenous sources. The authors have to first establish that the earthworms contain proteins only as the nitrogenous compound. Otherwise they can remove any mention about proteins only and rewrite the article for total nitrogen content.

Answer: The manuscript has been changed towards total nitrogen content. The word 'protein' has been changed to 'nitrogen' or 'total nitrogen content'

2. The use of conversion factor does not solve the issue, as the conversion factor refer to the nitrogen in proteins only. The authors should include the mechanism of the Kjeldahl method and its limitations need to be clearly mentioned. Discussions of these limitations on the final conclusions need to be included.

Answer: The response of this study has been changed to total nitrogen, not protein. Therefore, no more issues on conversion factor and limitation of Kjeldahl.

3. If the mechanism of the Kjeldahl method is analyzed then it would become clear that minimum digestion time and minimum amount of sulfuric acid are necessary for complete decomposition of the any nitrogenous compounds to ammonium sulphate.

Answer: The minimum amount of sulphuric acid and digestion time as suggested by the standard operating procedure has been included under Design of Experiment section.

4. Whether the authors have determined the minimum requirements of digestion time and sulphuric acid for this system should be mentioned. If these minimum conditions are not used then the conversion will be incomplete. This is mentioned towards end of the discussion in the paper, which implies that for some of their experiments conversion is not complete and the results become questionable. The authors claim their estimate of protein content (?) is higher than reported. An acceptable explanation for this difference should be included.

Answer: The minimum requirements for this system has been stated in the standard operating procedure of Kjeldahl Buchi manufacturer and do not need to be determined. Author has agreed to change their statement from protein to nitrogen as no specific quantification on protein nitrogen (PN) and nonprotein nitrogen (NPN) has been conducted. However, authors retain statement that using Kjeldahl showed some advantages over other methods.

5. The usage of Design Expert is incomplete and the authors should refer some publication in this direction and state clearly whether they have obtained optimum condition or not.

Answer: We claim that this is the first time Design of Expert software was used in the determination of nitrogen content from earthworm. See page 2 paragraph 2, final sentence:

“To the authors best knowledge, there has yet any papers published on the use of statistical tools (Design of Expert software) in the determination of protein content in earthworm powder by employing the Kjeldahl method of analysis. Utilization of statistical tools like Design Expert software offers many advantages.”

6. Quality of the graphs should be improved by improving the font size of the labels and the axes.

Answer: The qualities of the graphs have been improved. See Figure 1 and 2.