**Reference: [JSCS 9367]**

**Title: Prediction of denitrification capacity of alkalotolerant bacterial isolates from soil - an artificial neural network model**

**Comments to the Author**

**Reviewer F:**

Does the manuscript contain enough significant original material?: yes

Is the manuscript clearly and concisely written?: no

Are the conclusions adequately supported by the data?: yes

Does the manuscript give appropriate credit to related recent publications?: yes

Are the references appropriate and free of important omissions?: yes

Is the length of the manuscript appropriate?: yes

Does the manuscript need condensation or extension?: no

Is the quality of the figures (including legends and axes labelling) satisfactory?: yes

Are the nomenclature and units in accordance with SI?: yes

Are the English grammar and syntax satisfactory?: no

AUTHORS: The authors would like to thank the Reviewer for professional and helpful comments. It is obvious that the Reviewer is an expert in this field. The reviewer`s comments contribute to a better quality of the paper that was submitted. All remarks are accepted and paper is changed according to these comments.

**ADDITIONAL COMMENTS**

1. The meaning of the terms “denitrification kinetics” or “following denitrification kinetics”(?),

“denitrification capacity” and “bacterial behavior” is not clear and is sometimes confusing.

Thus, in the line 67 it is stated: “The aim of this study was to determine the denitrification capacity and bacterial behavior” implying that the two terms mean two separate things.

So, the lines 62-69 are worded as follows: “…were based on following denitrification kinetics: the biomass and N2 gas production, nitrate and nitrite concentration as well as nitrite and ammonia formation…Furthermore, the bacterial behavior during denitrification was investigated…

On the other hand, the lines 222-225 say as follows: “model satisfactorily predicted bacterial behavior during denitrification of all tested isolates during the denitrification process on(?) the turbidity and N2 gas formation kinetics, nitrate and nitrite concentration kinetics and nitrite and ammonia formation kinetics…” as if these two terms have the same meaning.

The contents of these terms should be clarified and used consistently.

For the same reason, lines 156, 191 and 230 should be made more precise.

The authors wish to express their gratitude for the Reviewer's observation. After a few readings of our paper, we decided to make a change in terminology for a better understanding of these terms. Considering that determination of denitrification capacity of soil bacterial strains by the ANN modelling was our research interest, correction in terminology was done.

Instead of “denitrification kinetics”, “following denitrification kinetics”, “bacterial behaviour during denitrification”, we uniformed terminology through all text with “denitrification capacity of the bacterial strains” and “denitrification indicators” for all outputs followed during the denitrification process (biomass and N2 gas production, nitrate and nitrite concentration, and nitrate and ammonia formation).

Once again, thanks to the Reviewer's observation for these confusing terms which resulting in imprecise explanations in the text.

2) It is not clearly explained what are the input parameters for the tested ANN model. In the section Statistical analysis, line 108, it is stated that “the type of the isolate and time are independent variables”, while in the line 229 “bacterial phenotypic trait and incubation time” are the two input variables. These terms should be standardized.

Thank You for these remarks. The terms “bacterial type” and “bacterial phenotypic trait” are corrected into “the bacterial strain” which we used in the rest of the text during the explanation of soil bacteria.

3) According to the reference 15 cited in the section Experimental, subsection Microorganisms, the bacteria used in this study are a kind of “leftover” from another experiment for which they have been selected and isolated according to the certain requirements. But, there is no reason why the procedure is not described in the present paper. Also, the bacteria had been submitted to phenotypic characterization. Why this is also not described in the present paper and, most importantly, why the phenotypic trait or traits used as the input for the present ANN model were not specified? In my opinion, it is important to reveal what traits enable the prediction of denitrifying capacity.

It was a pleasure to hear that You read our previous work about isolation and characterization of the bacterial strains which are used in this paper. After the suggestion, the main highlights about bacterial strains were added in the subsection Microorganisms. Furthermore, a brief explanation about differences between complete and incomplete denitrifiers, as well as specifics of *P. stutzeri* ATCC 17855 as complete denitrifying bacteria, was added in Results and Discussion.

4) In the section Global sensitivity analysis, it is concluded that bacterial phenotypic trait has the relative importance of 60,4% and the time the influence of 39,6% on output results. Are these values the same in predicting each of the six parameters comprising the denitrifying capacity?

AUTHORS: Global sensitivity analysis investigate the overall influence of the input variables (bacterial phenotypic trait and the duration of the process) on the observed outputs. According to this analysis, the bacterial strain was more influential in the calculation. The impact of the input variables on just one output variable could be also investigated using the global sensitivity analysis. In that case the effect of bacterial strain would still be stronger than the impact of time, ranging between 59.2% and 64.1%.

5) Lines 62-69 describing the main idea and the aim of the study should be re-written. They should make clear that the ANN model is being developed to predict bacterial denitrifying capacity (characterized by the biomass and N2 gas production, nitrate and nitrite concentration as well as nitrite and ammonia formation) based on bacterial phenotypic trait or traits and incubation time as independent inputs. Also, it should be explained that P. stutzeri ATCC 17588 was used as a REFERENCE microorganism known for its high denitrification capacity.

The authors would like to thank the Reviewer for this helpful comment. After complete revision, the aim of the paper was re-written and we hope that the main idea of our work is described in a better way. Also, we agree that we did not emphasize precisely why we use *P. stutzeri* ATCC 17588, so we added that part too.

6) The language is below the required standard. Some smaller corrections are introduced in the course of the review but there are many more mistakes. These should be carefully checked both in the text and in the table and figure captions.

The Manuscript was thoroughly checked, the noticed typing errors were corrected, by the English-speaking professional, according to the Reviewer’s suggestion.

7) Abbreviations TP and EA in the line 207 are not explained.

Thank You for this observation! We had made changes in terminology and removed these abbreviations for better understanding.

**REPORT:**

The paper presents an attempt to use an artificial neural network (ANN) modeling for prediction of the denitrifying capacity of bacterial isolates. The results show that such an ANN application yields reliable outputs that correlate with the experimental data. But the paper is written so imprecisely, that the explanations and conclusions are sometimes quite unclear. The points that need to be clarified are listed in a separate comment sheet.

AUTHORS: Thank You very much for the helpful comments and goodwill to help us to improve the Manuscript, according to the rigorous standard of the Journal of the Serbian Chemical Society

In my opinion, this manuscript should: be published after major revision and additional review

AUTHORS: The Authors decided to revise the manuscript according to the Reviewer’s remarks, believing that the changed paper would satisfy the Reviewer's criteria.