

Thank you very much for your appropriate indications and kind suggestions. In the revised version, the manuscript was improved according to your useful comments as follows.

As it is mentioned in the introduction, there is plenty of papers about biosensors for toxicity sensing, and I guess particularly using diuron, simazine, and atrazine at least... Could a few words be added to have a state of the art about the sensibility of the other biosensors to these compounds and thus, the motivation of designing a new biosensor?

→Detection limit for diuron of conventional reported biosensors were from 2.5 to 100ppb. Simple and low-cost environmental biosensors for eco-toxic compounds are strongly required in developing countries. Our motivations of designing a new biosensor were mentioned in the introduction of the revised manuscript. (See L.83-85)

In the material and methods part, chemicals are listed by a strange way (all together). Maybe it should be better organized by kind of products?

→In revised manuscript, only important chemicals are listed. Chemicals for μ CP process were mentioned in the explanation of each process. (See L.91-95)

In results and discussion part, the main difficulty for me is to replace the use of this biosensor in the context of all other existing biosensors dedicated to the sensing the pesticides studied here. In what this biosensor will bring something more robust or precise or performing than other already published biosensors? It is obviously due to the rapidity of the analysis, and it could more emphasized by a rapid comparison with the techniques classically employed in the text and perhaps also as an illustration (maybe in a Table format)?

→Detection limit for diuron of this biosensor is 1ppb. Detection limit for diuron of conventional reported biosensors were from 2.5 to 100ppb. Our biosensor showed higher sensitivity compared with conventional biosensors. Furthermore optical oxygen sensor chip was easily prepared by simple stamp process. Optical oxygen sensor chip can be easily measured by using a mobile phone. In the revised manuscript, these advantages of our biosensor were explained. (See L.393-402)

The same kind of question concerning the sensitivity of this biosensor for diuron (13 ppb) compared to what is found in the bibliography... Can the authors compare this value to the ones traditionally obtained by other biosensors? This could be added in the same Table (see my previous question).

→Detection limit for diuron of this biosensor is 1ppb. 13ppb as IC50 value in our original manuscript was the reported EC₅₀ value in literature. This is our mistake. Detection limit for diuron of conventional reported biosensors were from 2.5 to 100ppb. Our biosensor showed higher sensitivity compared with conventional biosensors. (See L.381-386, 393-397)

In none of the graphs there are standard deviations. Could you please add these indications? It will enhance the credibility of the results.

→We do not have sufficient data to show standard deviation. Therefore we cannot add error bars to the graph. Standard deviation of results for 9 spots in one biosensor chip was 11 – 12%. (See L.349-350)

I am not sure to understand the utility of Fig. 6. What does it imply exactly? Do you advise to use 2/2 min pulse?

→We tried to measure not only photosynthetic activity but also respiration activity, in order to change viable activity of algae during the use of the biosensor. Fig.6 imply repetitive measurements of O₂ production under light irradiation and O₂ consumption in dark. For this purpose, 2min with light irradiation and 2min without light irradiation was the suitable condition. (See L.317-320)

I don't really understand how the microalgae produce oxygen in the absence of light and in presence of diuron (red curve, Fig. 7)? A non-toxic case? At which concentration is the diuron in this case?

→Red curve in Fig.7 shows the result in the absence of light and diuron (Note in Fig.7 was not correct in original manuscript). And increase of fluorescence intensity means decrease of oxygen. Slight decrease of oxygen was caused by respiration of algae.

Pesticides were diluted with a solvent (dimethyl sulfoxide-DMSO). Thus, it appears necessary to perform controls with this compound in order to check that it has no negative impact on the microalgae photosynthesis and oxygen emission, and to be sure that the observed effects are only due to the pesticide alone. Clearly, why the authors did not add a curve of test with DMSO and without pesticide in Fig. 8?

→We tested several solvents and addition of 1% DMSO aqueous solution showed no effects on algae activity. Therefore we used 1% DMSO as a solvent. 0ppm of pesticides means only 1% DMSO was added. In Fig.8 of the original manuscript, 0ppm was printed as 0.0001ppm. In the revised manuscript, 0.0001 was corrected to 0. (See L.216-218)

It is not very clear from the material and methods section that the “pesticide free” sample used at time 0 was a blank for all along the experiment?

→Pesticide free sample was 1% DMSO aqueous solution. (See L.218)

The sentence L.352 to L.354 is stated for Fig. 8. It should be mentioned inside brackets, and the sentence should appear later in the text.

→The sentence L.352 to L.354 was moved to the place after “...are shown in Fig.8.” (See L.374-376)

The biosensor is presented as rapid test (15 min per sample, L.403). Therefore, I was wondering why the time-course experiment in Fig. 7 does not go until 15 min.

→15 min per sample(L403) was not correct. 10 min is correct. In Fig.7, before measurements the sensor chip was incubated with pesticides solution for 5 min in dark. Then fluorescence intensity change was measured under the light. Since Fig.7 shows the change became flat after 5min, 5min was selected as incubation time under the light. Therefore total incubation time of this sensor is 10min. (See L.372-374)

In relation to the previous question, it is not really clear for me, at which incubation time the data presented in the curves from Fig. 8 were obtained. Is it 5 min (like I suppose from the text in material and methods, L. 255), 10 or 15 min ? It is definitively not clear...

→ In Fig.8, incubation time (measurement time) for each pesticides was 10 min. A first the sensor chip was incubated with pesticides solution for 5 min in dark, then 5 min incubation under the light. (See L.372-374)

I strongly suggest the authors to read carefully the manuscript and perform extensive editing of English. There is too numerous English mistakes, first of all in the abstract and the introduction. All together, these grammatical mistakes strongly downgrade the paper. As examples, the first sentence of the abstract is too long, and I suggest to divide it in two parts (there are other examples all along the introduction).

→ The first sentence of the abstract was divided into two parts. (See L.10-14)

Some mistakes and corrections:

L.19 "...**the** biosensor showed..."

L.20 "... but **was** insensitive for..."

L.30 "...758 metric tons were **used**..."

L.40 "...**4** million people suffering..."

L.44 "... are found **now frequently** and **suspected** to have **a** direct link..."

L.71 "**Immobilization**" instead of "immobilizing"

L.298 "... that **helps to supply** light easily..."

L.340 "... as a **result** the oxygen generation rate is decreasing."

L.368 "**diuron**"

→ The suggested mistakes were corrected in the revised manuscript.