



SDI Review Form 1.6

PART 1:

Journal Name:	International Research Journal of Pure and Applied Chemistry
Manuscript Number:	MS: 2012/IRJPAC/2489
Title of the Manuscript:	COMPARATIVE STUDY OF BENZYL AND PHENYL COMPOUNDS EFFECTS ON THE INHIBITION OF METHANE GAS BIOSYNTHESIS

General guideline for Peer Review process is available in this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)

- This form has total 9 parts. Kindly note that you should use all the parts of this review form.



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PART 2: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<u>Compulsory</u> REVISION comments	The manuscript as a whole is an important study since the problem of degradation of organic wastes is topical. The authors investigate the inhibitory effect of some aromatic compounds on biological methane production. The problem studied in the manuscript needs to be definitely investigated and new obtained data are valuable. Nevertheless, the authors should clearly specify the relevance and the goals of the study, the main results obtained and their novelty compared to what is known in this research area. Otherwise, there is an impression that the most part of obtained results is to some extent a repetition of the works published already elsewhere. There are publications where similar studies were done and similar principle conclusions were made. The list of references contains many old papers. Is it really true that the main experimental works on the given problem have been done 15 or 20 years ago? On the basis of all mentioned above, the conclusions need clear a comparative analysis of obtained results with the emphasis of their contribution to the problem.	The manuscript is investigating the effect of aromatic compounds, known as xenobiotics and their structural chemistry on methanogenic bacteria is of great interest, not only to access their environmental impact but specially for the implementation of an efficient biodegradation process. Those organic compounds are widely used in industry and, therefore, the concern on their degradation dictates the development of efficient processes for their removal. Besides, it is also studying the structure-toxicity relationships. The ultimate goals is the modelling of the effect of aromatic structure on the inhibition of methane biosynthesis. The manuscript was revised to meet your preoccupations.
<u>Minor</u> REVISION comments	1. I would recommend to change the end of the title and use e.x. "of methane production (or formation) at the digestion of pig manure" instead of "methane gas biosynthesis" that looks a bit pretentious. 2. The term "methanogenic bacteria" does not exist anymore. Methanogenic microorganisms are now classified as archaea (not bacteria). It is also possible to use the term simply "methanogens". This correction	1. The end term of the title was changed in " of methane production by digested pig manure methanogens ". 2. In the past Archaea had been classed with bacteria as prokaryotes (or Kingdom Monera) and named archaebacteria, but this classification is regarded as out-dated. Currently they are called methanogens or archea (corrected in the



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	should be made throughout the text. 3. Page 2, Line 72. The important thing is to characterize the inoculum used for the experiment in detail. Some characteristics of the inoculum like the content of VFA, particularly acetate and methanogenesis rate in more common units e.x. mM/l as well as conditions of its digestion (except temperature) like pH and available nutrients, first of all acetate, should be specified in order to evaluate its potential to produce methane. How was acetoclastic methanogenic activity determined? Were acetate or any other nutrients used to maintain the digestion in the inoculum for five months? 4. Page 3, Line 80. Usually volatile fatty acid solution includes a mixture of at least acetate, propionate and butyrate. If acetate was the only substrate, then it would be reasonable to use "acetate" instead of VFA. Since high concentration of acetate can also inhibit methanogenesis, it would be also useful to present its concentration in more common units as mM. 4. Page 3. Line 128, 132. The use of the word "gas" after methane or CO₂ is not really necessary since it is clear that no biological activity possible at other states of those substances. 5. The IC₅₀ on the figure 2 is not correctly localized. The lines do not cross the curve in the proper point. 6. Since the experiments were done in triplicates, then the deviation intervals should be shown on the figure or indicated in the text. 7. Page 4. Line 152. "b" instead of "B". 8. The deviation intervals (numbers of digits after comma) in the table 1 should be corrected. They should be different for different values. 9. Page 5. Line 166. This phrase and the note to the figure 3 are identical. It should be changed. 10. Figure 3 contains CI50 meanings. Is it IC₅₀?	text). 3. - The characteristics of the inoculum (digested pig manure) are given in the manuscript. -The acetoclastic methanogenic activity was calculated from the slope of the cumulative methane production versus time curve and the quantity of VSS. - Only the additions of pig manure were used to maintain the digestion in the inoculum for five month. 4. The term VFA was removed in the text. 100 g COD-CH₃COOH/L = 1,562.5 mM of acetic acid 4. The term gas was deleted. 5. Corrected 6 Deviation intervals is showed on the figure. 7..Correted 8. corrected 9. Changed 10. CI₅₀ is IC₅₀ in French, corrected
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	11. Page 6. Line 232. It is written: “the obtained results are comparable to those reported...” As mentioned above, it is important to clearly identify the new contribution of the obtained results compared to the previous ones. 12. Page 7. Line 250. The statement can be accepted only if the deviation intervals are known. The meanings of IC₅₀ for two compounds are very close. 13. Page 8. Line 298-300. Experiments with compounds having Cl- and Br-substitutive groups look interesting, but not completed. More data on the experiments with other Cl- and Br-compounds or aromatic compounds of another structure could strengthen the manuscript.	11 Corrected 12. The IC₅₀ values in figure 4 are in log scale. See the IC₅₀ values in table 1, 146.65 and 208.78 respectively for toluene and benzene. 13. We have studied about 70 aromatic compounds but the only benzyl and phenyl compounds having Br-substitutive are given in this paper. But we have studied many Cl-aromatics. The aliphatic compounds are not in general toxic to methanogens. We are studying the effect of aromatic chemical structure on inhibition of methane production,. If we give the data of other Cl-aromatics, the structure comparison would not be made as stated in the title.																		
<u>Optional/General</u> comments	The study looks incomplete in its present form. It requires corrections in the general style with emphasis on main issues of the study. It would be highly reasonable to provide the manuscript with additional results. Some data on tests of other compounds as inhibitors of methanogenesis and further analysis would make the story more comprehensive.	<table><tr><td>Compounds</td><td>IC₅₀ (mg/L)</td></tr><tr><td>m-Dichlorobenzene</td><td>44.1±4.1</td></tr><tr><td>o-Chloroaniline</td><td>51.0±3.2</td></tr><tr><td>p-Chlorothiophenol</td><td>638.3±29.6</td></tr><tr><td>m-Chloroaniline</td><td>539.8±3.4</td></tr><tr><td>p-Chloroaniline</td><td>787.5±20.4</td></tr><tr><td>anisole</td><td>168.8±5.3</td></tr><tr><td>salicylic acid</td><td>987.0±24.9</td></tr><tr><td>salicylic aldehyde</td><td>1002.3±7.2</td></tr></table>	Compounds	IC ₅₀ (mg/L)	m-Dichlorobenzene	44.1±4.1	o-Chloroaniline	51.0±3.2	p-Chlorothiophenol	638.3±29.6	m-Chloroaniline	539.8±3.4	p-Chloroaniline	787.5±20.4	anisole	168.8±5.3	salicylic acid	987.0±24.9	salicylic aldehyde	1002.3±7.2
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