



SDI Review Form 1.6

PART 1:

Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	MS: 2012/IJBcRR/2458
Title of the Manuscript:	EFFECT OF PARA SUBSTITUTED ANILINES CHEMICAL STRUCTURE ON METHANE BIOSYNTHESIS BY THE METHANOGENS

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.



SDI Review Form 1.6

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)
Compulsory REVISION comments	Dear Authors, The present work reports the "EFFECT OF PARA SUBSTITUTED ANILINES CHEMICAL STRUCTURE ON METHANE BIOSYNTHESIS BY THE METHANOGENS". This type of work is definitely important for waste water treatment plant. They have done Basic research work and the quality of the paper is very poor. The methanogenic activity/anaerobic activity test of an organic molecule is the one parameter to judge the anaerobic biomass is active on that concentration or environment. If your Biomass is good or susceptible on that concentration then you should go for biodegradation of any molecule (p-Nitroaniline, p-Chloroaniline, p-Toluidine etc..) by this biomass. Then you report that this pig manure biomass is very efficient for biodegradation of this toxic molecule and the toxicity effect of that organic molecule. After I got your revised manuscript on 7 th Jan, 2013 I have done personally methanogenic activity of aniline. Now I have seen that my results also coincide the references* (<10 mM for aniline ie, <940 mg/l ^{###}). My biomass was granule biomass from the local wastewater treatment plant. I think you have done some mistake on this research. Please reproduce the experiment and read this article very carefully and then you get better idea about this.	1. In the first version of this manuscript we wrote in introduction that: " Para substituted anilines compound as the aromatic compounds can be biodegraded in anaerobic digestion process but they can also be toxic to methanogenic bacteria". Indeed, in our research on modeling the effects of aromatic structure on the inhibition of biogas biosynthesis, the methanogenic toxicity of nitroaromatic and aminoaromatic compounds has been investigated with digested pig manure as inoculum and the types of toxicity caused by these compounds on the methanogenic bacteria of the inoculum was also studied. The nitroaromatics was found many time toxic than aminoaromatics. According to Donlon et al. [5], Nitroaromatics were clearly very toxic compounds to methanogens. Aromatic amines, in contrast, were less inhibitory. Nitroaromatic compounds were, on average, 500-fold more toxic than their corresponding aromatic amine analogs. But in our studies, Nitroaromatic compounds were, on average, 335-fold more toxic than their corresponding aromatic amine analogs. During the anaerobic toxicity assay, it was noticed

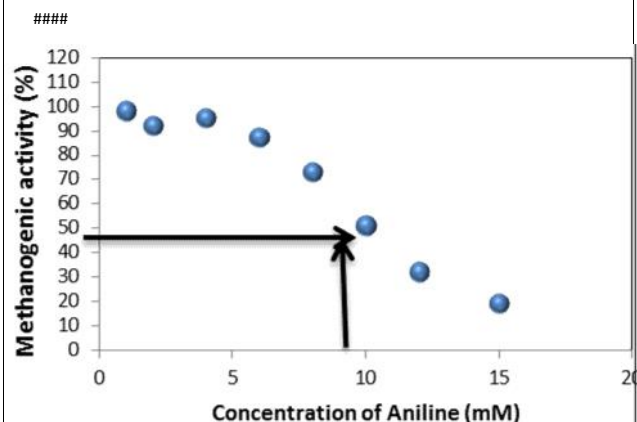


SDI Review Form 1.6

1. Angelidaki, M. Alves, D. Bolzonella, L. Borzacconi, J. L. Campos, A. J. Guwy, S. Kalyuzhnyi, P. Jenicek and J. B. van Lier (2006). Anaerobic Biodegradation, Activity and Inhibition (ABAI) Task Group Meeting. 9 to 10 October 2006, Prague (Czech republic).

2. Coates, JD; Coughlan, MF; and Colleran, E (1996). Simple method for the measurement of the hydrogenotrophic methanogenic activity of anaerobic sludges. *Journal of Microbiological Methods*, 26, 237-246.

3. Colleran, E; Concannon, F; Golden, T; Geoghegan, F; Crumlish, B; Killilea, E; Henry, M; and Coates, J (1992). Use of methanogenic activity tests to characterize anaerobic sludges, screen for anaerobic biodegradability and determine toxicity thresholds against individual anaerobic trophic groups and species. *Water Science and Technology*, 25 (7), 31-40.



* http://www.uasb.org/SCIENCE/data_page_toxicity.htm

that the nitroaromatics were biodegraded in aminoaromatics when the inhibition of nitroaromatics caused a metabolic toxicity to methanogens but when the inhibition of methanogenic activity was higher than 75 %, the nitroaromatic toxicity was generally bactericidal and sometimes physiological. The ability of methanogenic archaea to remove and detoxify the nitro group in nitroaromatics would make anaerobic processes a useful treatment adjunct and/or alternative to conventional aerobic systems. In particular, anaerobic nitro group reduction may be important initial step, which when followed by aerobic post treatment, could result in complete mineralization of such highly nitrated compounds as trinitrotoluene and picric acid which are highly resistant to aerobic biodegradation (K. Kayembe et al, unpublished data).

2. Aniline IC₅₀ Value

a) We also repeated the anaerobic toxicity assays of aniline with digested pig manure and with a granular sludge from an anaerobic wastewater treatment plant, treating effluents from a brewery (Breda/The Netherlands). The results are as follows,

IC₅₀ with granular sludge: 885.76 ±23. 86 mg /l;

IC₅₀ with pig digested manure: 1364.69±29.30 mg /l .

On the one hand, our value with granular sludge



SDI Review Form 1.6

		coincide with the value found by J. Field and R. Sierra (http://www.uasb.org/SCIENCE/data_page_toxicity.htm) and on the other hand, the value with pig digested manure remains the same. c) According to J. Field and R. Sierra [6], the IC₅₀ values depend of substrate nature, type of inoculum, pH, experimental temperature, acclimation, etc. Would you please, determine the IC₅₀ value of aniline with pig manure as inoculum for comparison with our value? d) In addition, our IC₅₀ values have been accepted in a lot of international journals including the IC₅₀ value of aniline [9;10,19,20].
<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments		