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Effect of Chloroaniline Isomerism on Inhibition of Methane Biosynthesis by the Methanogenic Bacteria

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Authors' contributions

This work was carried out in collaboration between all authors. Author KK designed the study and performed the experimental work, author LB performed the statistical analysis, wrote the protocol; author PTM wrote the first draft of the manuscript, managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The present work aims to determine the toxicity of Chloroanilines isomers and evaluate the effect of Chloroanilines isomerism and intramolecular hydrogen bonds on inhibition of methane biosynthesis by the acetoclastic methanogenic bacteria.
Study Design: Anaerobic digestion of pig manure, anaerobic toxicity essay, Effect of the isomerism (functional groups position) on inhibition of methane biosynthesis by the methanogenic bacteria), Correlation of the methanogenic toxicity (IC_{50}) with Chloroanilines boiling point.
Place and Duration of Study: Department of Chemistry, University of Kinshasa, DR Congo, between April 2011 and March 2012.
Methodology: The toxicity to acetoclastic methanogenic bacteria was performed with the standard method of serum bottles; digested pig manure was utilized as inoculums and acetate as substrate. The methane gas volume produced was measured by serum bottles liquid displacement systems (Mariotte flask system).
Results: The obtained results indicate there are relationships between the isomerism of Chloroanilines and their inhibitory effects on methanogenic bacteria. The various isomers of Chloroaniline have toxicities which are different from 51.00; 539.78 and 787.47 mg / l respectively for the ortho, meta and para-Chloroaniline. One can notice that the o-Chloroaniline IC_{50} value is 10.43 and 15.44 times more toxic than meta and para

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Chloroaniline respectively. Contrary to all predictions, it is more toxic than benzene. This behavior can be explained by the formation of a strong hydrogen bond between the electronegative chlorine atom (-Cl) and amino group (-NH_2) in o-Chloroaniline in ortho position of aromatic ring. The formation of this intramolecular bond decreases the steric hindrance of this isomer and increases its mobility (diffusion coefficient) to the cell membrane). A significant linear correlation was found between the IC_{50} values of Chloroanilines isomers and their boiling temperatures.

Conclusion: The results obtained in this paper indicate that some relationships exist between the isomerism (functional groups position) of Chloroanilines and their inhibitory effects on methane biosynthesis by the methanogenic bacteria. The formation of a very strong intramolecular hydrogen bonds between chlorine atom and amine group renders ortho Chloroaniline very toxic than the others tested Chloroaniline isomers.

Keywords: Chloroanilines; hydrogen bond basicity (HBB); methane biosynthesis; isomerism; inhibition; methanogenic bacteria; boiling point.

1. INTRODUCTION

Aniline aromatic substance, used in the 19th century for the production of dyes (mauvéine) and drugs, then extracted from natural substances like indigo. Also called phenylamine or aminobenzene it is currently obtained by chemical process from nitrobenzene. Chloroanilines are obtained from the chlorination of aniline precursors: three isomers of chloroaniline, six isomers of dichloroaniline, four isomers trichloroaniline. Bioaccumulation of these aromatic compounds is generally low; they are under certain conditions degradables in the environment (photolysis) except in soils and sediments. Aniline and chloroanilines are toxic to humans and very toxic to the environment (especially aquatic). Impact on the respiratory activity by converting the hemoglobin, generalized disorders induced with potential irreversible effects, possible carcinogen and absorbed through skin [1].

4-Chloroaniline is used as an intermediate in the production of several urea herbicides and insecticides (e.g., monuron, diflubenzuron, monolinuron), azo dyes and pigments, and pharmaceutical and cosmetic products. In 1988, about 65% of the global annual production was processed to pesticides (e.g. 4-chloroaniline). The 4-chloroaniline based azo dyes and pigments are especially used for the dyeing and printing of textiles. Triclocarban is a bactericide in deodorant soaps, sticks, sprays, and roll-ons, and chlorohexidine is used in mouthwashes and spray antiseptics. 4-Chlorophenol is also listed as an antimicrobial agent for cosmetic products in the European Inventory of Cosmetic Ingredients. However, no information is available on the products in which 4-chloroaniline is used. All of these products may contain residual 4-chloroaniline, or 4-chloroaniline may emerge during their degradation [1].

The chloroanilines are used for the industrial manufacture of many products: Isocyanates, polyurethane intermediates, antioxidants and accelerators of vulcanization (rubber industry), Industry of dyes, pesticide products (diuron, linuron), pharmaceuticals and chemicals products. Aniline and chloroaniline like all aromatic compounds can be biodegraded in anaerobic digestion process but they can also be toxic to methanogenic bacteria and inhibiting the methane biosynthesis. Literature on anaerobic digestion shows considerable variation in the inhibition/toxicity levels reported for most substances. The major reason for these variations is the complexity of anaerobic digestion process where mechanism such as antagonism, synergism, acclimation and complexing could significantly affect the inhibition

phenomenon [3,13,10]. The present work aims to determine the toxicity of chloroanilines isomers and evaluate the effect of isomerism and intramolecular hydrogen bonds on the chloroanilines inhibition of methane biosynthesis by the acetoclastic methanogenic bacteria.

2. MATERIALS AND METHODS

2.1 Biomass

Pig manure from the Nsele Presidential Agro-Industrial Domain (DAIPN) farm of Nsele /KINSHASA (DR CONGO) was digested in laboratory scale digester during about nine months. The digested pig manure (sludge) was utilized as inoculums in our anaerobic toxicity tests. The digested ping manure was not previously acclimated to any aromatic compounds. Characteristic of inoculums: total suspended solids (TSS) concentration 91.10 g/L, volatile suspended (VSS) concentration 56.59 g/L, specific acetoclastic methanogenic activity 163.40 -210.81 mg COD-CH4/g VSS .d (27±1°C) .

2.2 Basal Medium

The basal medium used in the anaerobic toxicity assay contained the following : $NaHCO_3$, 5,000 mg/L; NH_4Cl, 280 mg/L; $CaCl_2.2H_2O$, 10 mg/L; K_2HPO_4, 250 mg/L; $MgSO_4.7H2O$, 100 mg/L; yeast extract,100 mg/L; H_3BO_3,0.05 mg/L; $FeCl_2.4H_2O$, 2 mg/L; $ZnCl_2$, 0.05mg/L; $MnCl_2.4H2O$, 0.05 mg/L; $CuCl_2.2H_2O$, 0.03 mg/L; $(NH4)SeO_3.5H_2O$, 0.05 mg/L; $AlCl_3.6H_2O$, 2 mg/L; $NiCl_2.6H_2O$, 0.05 mg/L; $Na_2SeO_3.5H_2O$, 0.1 mg/L; EDTA, 1 mg/L; resazurin, 0.2 mg/L; as well as 36% HCl at 0.001ml/L, pH 7.2 [5,7].

2.3 Aromatic Compounds

The three isomers of Chloramines were all used: ortho chloraniline, Meta chloraniline and para chloramines. Otherwise benzene, chlorobenzene and aniline were used as reference aromatic compounds. All aromatic compounds were p.a. products supplied by MERCK. The chemical structures of these aromatic compounds are given in the Fig. 1.

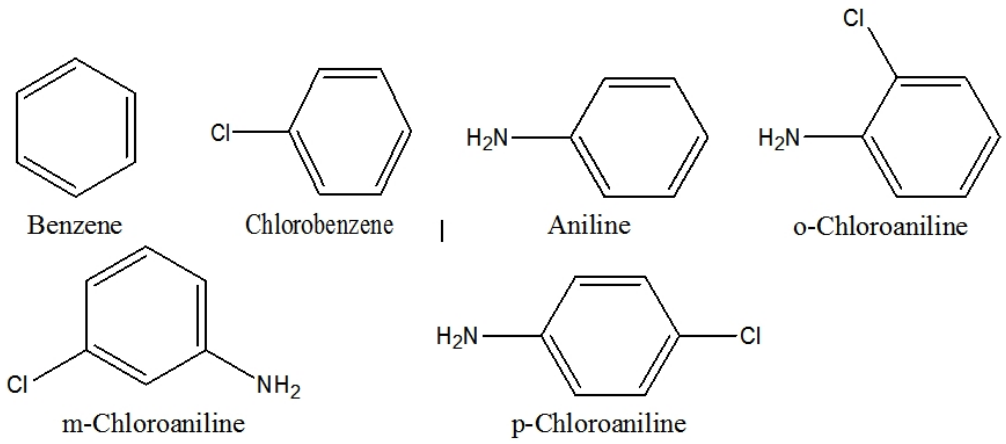

Fig. 1. The structures of chloroanilines and reference aromatic compounds

2.4 Anaerobic Toxicity Assay

Specific acetoclastic methanogenic activity measurements were performed with 1l glass serum bottles sealed with butyl rubber septa. Digested pig manure 1.5 g volatile suspended solids per liter (VSS/l) was transferred into glass serum bottles containing 900 ml of basal medium and acetate from a neutralized stock solution (pH 7) to yield a final concentration of 4g chemical oxygen demand per liter (COD)-CH3COONa/l. The required quantity of inhibitory compounds was added to each flask to provide the toxic concentration to be investigated. No toxicant was added to the controls. The toxicant concentration was chosen to cause an inhibition of the acetoclastic activity ranging from 0-100 % [14,15]. The concentrations of inhibitors used in the anaerobic toxicity assay are given in the Table 1.

Table 1. The inhibitory concentrations of aromatic compounds used in anaerobic toxicity assay

Nº	Compounds	Concentrations (mg/l)					
		1	2	3	4	5	6
1	Chlorobenzene	0	15	50	100	150	200
2	Benzene	0	150	300	450	600	750
3	o-Chloroaniline	0	15	30	60	90	120
4	m-Chloroaniline	0	300	700	1,500	2,000	2,500
5	p-Chloroaniline	0	500	1,500	2,000	2,500	3,000
6	Aniline	0	1000	2,000	3,000	4,000	4,500

The liquid was flushed with nitrogen gas for ten minutes and the flasks were sealed with rubber septum cap and placed in a reciprocating shaker at 27± 1ºC (room temperature). The specific methanogenic activity was calculated from the slope of the cumulative methane production versus time curve and the quantity of VSS. The compound concentration that caused 50% inhibition of the methanogenic activity had referred to as “50% IC”. All specific methanogenic activity measurements were conducted in triplicate. To determine the degree of inhibition, the specific methanogenic activities of the control and samples containing inhibitory compounds were determined [6,7,11].

2.5 Methane Gas Measurement

The methane gas volume produced was measured by serum bottle liquid displacement systems (Mariotte flask system) as previously described [6,11,13]. The liquid in the displacement serum bottle should contain a concentrated solution of NaOH or KOH in order to rapidly convert CO2 gas to carbonate and dissolve it into the NaOH solution [6].

3. RESULTS AND DISCUSSION

3.1 Effect of the Isomerism (Functional Groups Position) on the Inhibition of Specific Methanogenic Activity

The inhibitory effect of aniline isomers and reference aromatic compounds (benzene, Chlorobenzene and aniline) on the activity of acetoclastic methanogenic bacteria were tested at various levels, from the nontoxic concentrations to those that are completely inhibitory to acetoclastic methanogenic activity. The evolution of methanogenic activity is typified for the aniline in Fig. 2

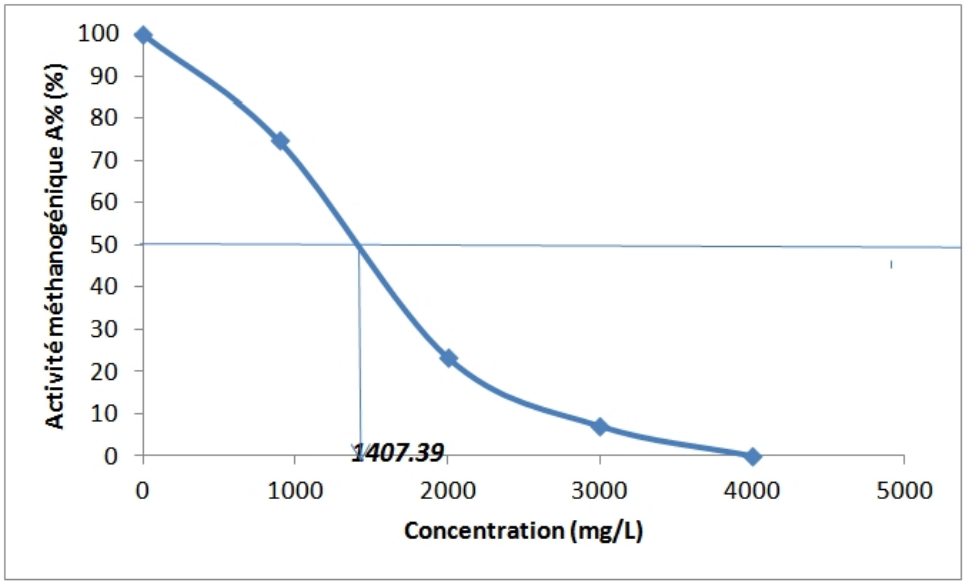

Fig. 2. Methanogenic activity of digested pig manure exposed to aniline as a function of the aniline concentration and IC_{50} determination

Fig. 2 shows the decrease in specific methanogenic activity with increasing the concentration of aniline and the IC_{50} values determination.

All concentrations of aromatic compounds used exerted an inhibitory effect on the specific acetoclastic methanogenic activity. This implies that these aromatic compounds are inhibitory to methane biosynthesis and some of them are toxics even in very small quantity. The Table 2 summarizes the 50% inhibiting concentrations (IC_{50}) of aromatic compound evaluated in this study, ranked in decreasing order of toxicity.

Table 2. The IC_{50} values observed in the study for the aniline and reference aromatics

N°	Compounds	IC_{50} (mg/l)	logPoct	b.p.(°C)
	Chlorobenzene	30.80±2.01	2.84	132.22
2	o-Chloroaniline	51.00±3.20	1.91	208.80
3	Benzene	208.78±6.32	2.13	80.10
4	m-Chloroaniline	539.78±3.42	1.90	226.00
5	p-Chloroaniline	787.47±20.43	1.87	232.00
6	Aniline	1407.39±22.91	0.90	184.68

Legend: b.p: Boiling point
logPoct: logarithm of partition coefficient water-octane

The results obtained indicate that relationships exist between the nature of aromatic compounds and their inhibitory effects on methanogenic bacteria. According to the Fig. 2, the toxicity of Chloroanilines and reference compounds increases, respectively, in the following order:

Aniline < p-Chloroaniline < m-Chloroaniline < Benzene <o-Chloroaniline < Chlorobenzene

In this sequence of toxicity, Chlorobenzene with 30.80 mg/l IC_{50} value is the most toxic compound, while the Aniline with 1407.39 mg/l is the less toxic. The substitution of an amino group (-NH_2) on the benzene ring, giving the Aniline, reduces the toxicity of the benzene ring while that of the Chlorine atom (-Cl), giving Chlobenzene, increases the toxicity of benzene ring. The grafting of Chlorine (-Cl) at Aniline to form bifunctional compounds that are Chloroanilines, make Chloroanilines to be more toxic than Aniline. And secondly, by adding an amino group (-NH_2) at Chlorobenzene to form Chloroanilines, these compounds become less toxic than Chlorobenzene. It can be noticed that o-Chloroaniline is most toxic than benzene.

With few exceptions, the results obtained in this study are comparable to those obtained in our previous work (Kayembe et al in press) and by Sierra and Lettinga, (1989) [6] for monosubstituted and bisubstituted aromatics. The addition of a functional group containing an oxygen, sulfur or Nitrogen heteroatom to benzene, our reference compounds, decreased the benzene toxicity as the case of amino (–NH_2) substitution. However, the addition of Chlorine atom (-Cl) to benzene was associated with an increase in compound toxicity.

This demonstrates that the grafting of hydrophobic or hydrophilic substituent on the benzene or monofunctional aromatic compound, make the obtained compound more or less toxic as the case and that in the same order of toxicity [4]. However, this behavior is only valid when the two substituents have not electronic or steric interactions and no formation of intramolecular hydrogen bonds. This is possible, when the two substituents are in the para position relative to each other. Indeed, when the substituents are in ortho or meta position, interactions change the order of toxicity in one direction or another (Kayembe et al. in press). This phenomenon can be interpreted by the fact that the toxicity of isomers varies with the position of functional groups that result in steric and electronic interactions, and also with the formation of intramolecular hydrogen bonds [4,9].

3.2 Effect of Intramolecular Hydrogen Bonds Formation on Chloroanilines Isomers Methanogenic Toxicity

The isomers of aromatic compounds can give us information on the influence of the substituents position on the methanogenic toxicity. Indeed; aromatic isomers have two identical substituents but differ only by their positions on the aromatic ring. The IC_{50} values of chloroanilines are compared in Fig. 3.

Indeed, despite the fact that the three Chloroanilines have identical substituents and the same molecular weight, they have different boiling point, IC_{50} and logPoct (Table 2). This implies that the isomerism has an influence on the physico-chemical properties of aromatic compounds as it is the case of Chloramines. In fact, according to the results reported in Fig. 3, the toxicity of various positional isomers of Chloroaniline is decreasing in the following order: o-Chloroaniline < m-Chloroaniline< p-Chloraniline.

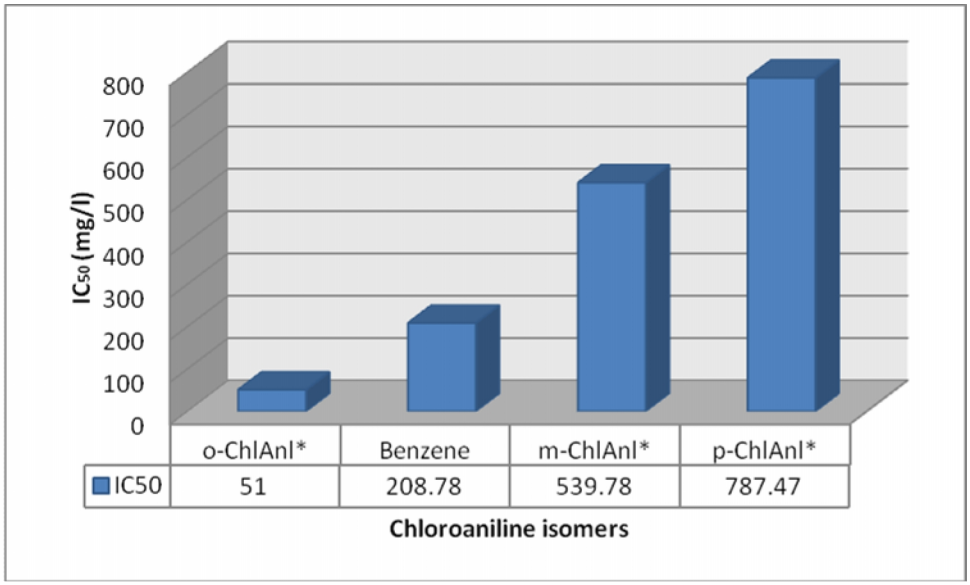

Fig. 3. Methanogenic toxicity comparison of chloroaniline isomers
** o-ChlAnl = o-Chloroaniline, m-ChlAnl = m-Chloroaniline, p-ChlAnl = p-Chloroaniline*

The various isomers of Chloroaniline have toxicities which are different from 51.00 mg / l; 539.78 mg / l and 787.47 mg / l respectively for the ortho, meta and para-Chloroaniline. One can notice that the o-Chloroaniline IC_{50} value is 10.43 and 15.44 times more toxic than meta and para chloroaniline respectively. Contrary to all predictions, it is more toxic than benzene. This is the consequence due to the introduction of the chlorine atom (-Cl), electron-withdrawing (inductive), in ortho position to the amino group ($-NH_2$) of aniline and the possibility of forming a very strong intramolecular hydrogen bond, significantly reduces the "hydrogen bond basicity (HHB)" of Aniline. The aromatic compound so formed (o-Chloroaniline) becomes more toxic (lipophilic) than the latter.

On the contrary, the substitution of chlorine atom (-Cl) in meta or para position of the NH_2 group of aniline decreases its "hydrogen bond basicity" (HBB). Aromatic compounds so formed (meta and para-Chloroaniline) become less toxic (less lipophilic) than o-Chloroaniline and more toxic than Aniline [4]. One can notice that the o-chloroaniline is 10.43 and 15.44 times more toxic than meta and para chloroaniline respectively. Contrary to all predictions, it is more toxic than benzene. This behavior can be explained by the formation of a strong hydrogen bond between the electronegative chlorine atom (Cl) and amino group ($-NH_2$) in o-chloroaniline [4,9].

All of our data from our research in the field of aromatic toxicity show that para substituted isomers are always less toxic than other the isomers while the ortho are most toxic (Kayembe et al, unpublished data). This behavior is valid only when there are no steric effects. Indeed, when a «basic » substituent is bonded to a benzene ring, the conjugation tends to reduce the basicity, resulting in increased lipophilicity relatively to the aliphatic compound. If you place a bulky substituent ortho to the basic substituent, it is more difficult to place in in the plane of the ring. It follows the decreasing of the conjugation thus increasing of the basicity and thus the hydrophilicity. The formation of this intramolecular

bond decreases the steric hindrance of this isomer and increases its mobility (diffusion coefficient in the cell membrane) [4,14].

3.3 Correlation of the Methanogenic Toxicity (IC_{50}) with Chloroaniline Boiling Point (Bp)

It was noticed that in many cases, the values of the ortho isomers logPoct and meta are equal as it can be seen for the ortho and meta Chloroaniline (Table 2). But, their boiling temperatures are different. Thus, we tried to know if a correlation could exists between the toxicity of Chloroanilines and their boiling temperatures.

Boiling points are 208.8, 226.0 and 232.0ºC respectively for ortho-Chloroaniline, meta-and para-Chloroaniline. It should be noted that the first two isomers (ortho-and meta-Chloroaniline) are liquid while (para-Chloroaniline) is solid. The inhibitory effects of Chloroaniline isomers have been assessed to study whether relationships between Chloroaniline boiling point and methanogenic toxicity could be established. The IC_{50} values of Chloroaniline isomers were plotted against the boiling temperatures of the Chloroaniline aromatic compounds. The Fig. 4 shows the correlation line between the methanogenic toxicity and boiling points for Chloroaniline isomers.

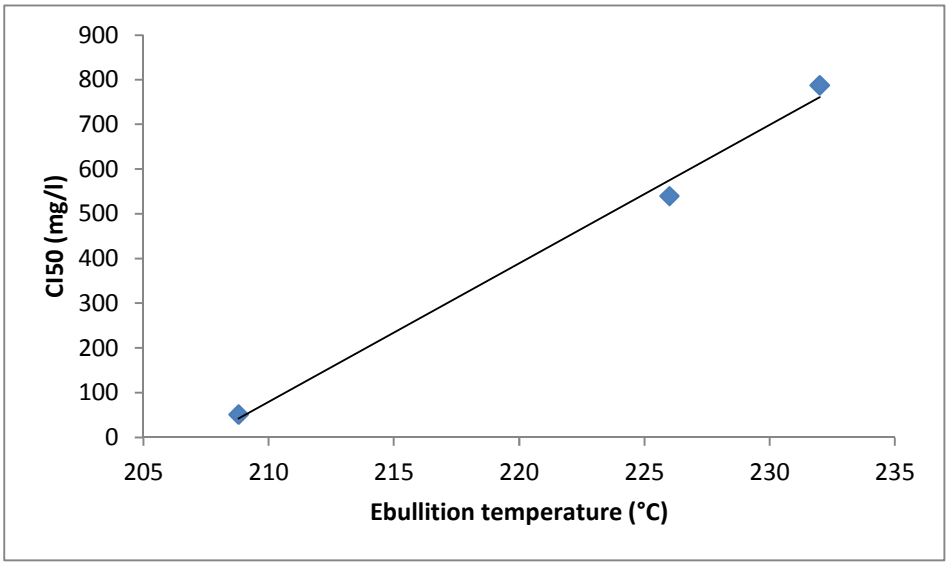

Fig. 4. Methanogenic toxicity (IC_{50}) with Chloroaniline isomers boiling point (ºC) correlation (R^2 = 0.9928)

A significant positive linear correlation had been observed between the IC_{50} values of Chloroanilines isomers and their boiling temperatures (R^2 = 0.9928). This implies that the order of isomer toxicities could be predicted from boiling point of aromatic isomers. But, any correlation between the IC_{50} values of Chloroaniline isomers and logPoct was not found. To our knowledge, this is first time that a correlation is found between the aromatic compounds methanogenic toxicity and the boiling temperature. Several of our data show that there are also such correlations in several series of the aromatic compounds structurally related (KAYEMBE et al. in press).

4. CONCLUSION

The obtained results indicate that relationships exist between the isomerism of Chloroaniline and their inhibitory effects on methanogenic bacteria. This demonstrates sufficiently that the grafting of hydrophobic or hydrophilic substituent on monofunctional aromatic compound, make the obtained isomeric aromatic compounds more or less toxic. The toxicity of various positional isomers of Chloroaniline is decreasing in the following order: o-Chloroaniline < m-Chloroaniline < p-Chloroaniline. All of our data from our research in the field of aromatic toxicity show that para substituted isomers are always less toxic than other the isomers while the ortho are most toxic.

One can notice that the o-Chloroaniline is 10.43 and 15.44 times more toxic than meta and para Chloroaniline respectively. Contrary to all predictions, o-Chloroaniline is more toxic than benzene. This behavior can be explained by the formation of a very strong hydrogen bond between the electronegative chlorine atom (Cl) and amino group ($-NH_2$) in o-Chloroaniline.

A significant positive linear correlation had been observed between the IC_{50} values of Chloroaniline isomers and their boiling temperatures ($R^2 = 0.9928$).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Booth G. Nitro compounds, aromatic. In: Ullmann's encyclopedia of industrial chemistry, John Wiley & Sons: New York; 2007. doi:10.1002/14356007.a17_411
2. Hecht C, Greihl C. Investigation of the accumulation of aromatic compounds during biogas production from kitchen waste. Bioressource Technology. 2009;100(2):654-658.
3. Chen Y, Cheng JJ, Creamer KS. Inhibition of anaerobic digestion process: A review. Bioressource Technology. 2008;99(10):4044-4064.
4. Debord J. “Coefficient de partage,” accessed : 15 April 2012. Available: www.unilim.fr/pages-peso.
5. Donlon BA, Razo-Flores E, Field JA, Lettinga G. Toxicity of N-substituted aromatics to acetoclastic methanogenic activity in Granular Sludge. App. Environ. Microbial. 1995;61(11):3889-3893.
6. Field J, Sierra R. Waste characteristics and factors affecting reactor performance. IHE-Delft & WAU, WAGENINGEN; 1990.
7. Kalyuzhnyi S, Sklyar V, Mosolova T, Kucherenko I, Russkova JA, Degtyaryava N. Methanogenic biodegradation of aromatic amines. Water Science and Technology. 2000;42(5-6):383-370.
8. Kobayashi K. Metabolism of pentachlophenol in fish. In: pesticide and xenobiotic Metabolism in Aquatic Organism, American Chemical Society, Symposium series. 1979;99.
9. Leo A, Hansch C, Elkins D. Partition coefficients and their uses. Chem Rev. 1971;71(6):525–616. doi:10.1021/cr60274a001.
10. Lettinga G, Hulshoff Pol LW. Anaerobic reactor technology, IHE-Delft & WAU, WAGININGEN; 1990.

11. Mambanzulua N, Kayembe K, Noki V. Détermination des activités méthanogènes spécifiques des lisiers dans le traitement anaérobique des déchets. Med. Fac. Landbouww. Univ. Gent. 1999;64(1):183-188. French.
12. Mbuyu K, Kayembe K. Etude de la toxicité méthanogénique des cations K^+ et Na^+. Rev. Cong. Sci. Nucl., 2000;16(2):65-76. French.
13. Mbuyu K, Kayembe K. Production du méthane (biogaz) par la digestion anaérobie des drêches de brasseries et des lisiers de vaches. Rev. Cong. Sci. Nucl. 2000;16(2):47-62. French.
14. Mpiana P. Biophysique médicale. Resud Edition, Kinshasa; 2010. French.
15. Sierra R, Lettinga G. The role of aromatic structure on methanogenic toxicity. Med Fac. Landbouww. Rijksuniv. Gent. 1989;4(4b):1437-1474.

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