

COMPARATIVE STUDY OF BENZYL AND PHENYL COMPOUNDS EFFECTS ON THE INHIBITION OF METHANE GAS BIOSYNTHESIS

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ABSTRACT

Aims: To determine the inhibitors effects of benzyl and phenyl compounds in anaerobic digestion process and comparing their inhibition on methane biosynthesis.

Study design: Anaerobic digestion of pig manure, anaerobic toxicity essay, comparison of benzyl and phenyl compounds effects on the inhibition of methane gas biosynthesis.

Place and Duration of Study: Department of Chemistry, University of Kinshasa (DR Congo), between September 2011 and May 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 the methane gas volume produced was measured by serum bottles liquid displacement systems (Mariotte flask system).

Results: The obtained results indicate that some general relationships exist between the nature of benzyl and phenyl compounds and their inhibitory effects on biosynthesis of methane by methanogenic bacteria. According to these results, α -chlorotoluene and bromobenzene with 0.61 and 2.90 mg/l as IC_{50} values, respectively, are the most toxic compounds, while phenol and benzyl alcohol with IC_{50} values of 1248.90 and 2391.37 are less toxic.

Comparing the phenyl and benzyl compounds with the same substituents their behavior was diverse. The benzyl chloride is more toxic than phenyl chloride and benzyl bromide is less toxic than phenyl bromide. Otherwise, benzyl alcohol is less toxic than phenol.

A significant correlation between the toxicity of phenyl compounds and their hydrophobicity ($R^2 = 0.911$) was found. But any correlation was not found for benzyl compounds

Conclusion: The obtained results indicate that relationships exist between the nature of benzyl and phenyl compounds and their inhibitory effects on biosynthesis of methane by methanogenic bacteria.

Keywords: Benzyl, Phenyl, biosynthesis, anaerobic digestion, toxicity, methanogenic bacteria

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1. INTRODUCTION.

In organic chemistry, benzyl is the substituent or molecular fragment possessing the structure $C_6H_5CH_2-$. Benzyl features a benzene ring attached to a CH_2 group. The term benzyl refers most commonly to benzyl compounds, such as benzyl chloride or benzyl benzoate. Sometimes, benzyl and phenyl are confused, but their formulas and behavior are very different. The term benzylic refers to the position on a carbon skeleton next to a phenyl or other aromatic ring (Carey and Sundberg, 2008).

Besides, the phenyl group or phenyl ring is a cyclic group of atoms with the formula C_6H_5 . Phenyl groups are closely related to benzene. Phenyl groups have six carbon atoms bonded together in a hexagonal planar ring, five of which are bonded to individual hydrogen atoms, with the remaining carbon bonded to a substituent. Phenyl groups are pervasive in organic chemistry. Although often depicted with alternating double and single bonds, phenyl groups are chemically aromatic and show nearly equal bond lengths between carbon atoms in the ring.

Phenyl groups are generally attached to other atoms or groups. Many or even most phenyl compounds are not described with the term "phenyl". For example the chloro derivative C_6H_5Cl is normally called chlorobenzene, although it could be called phenyl chloride.

Phenyl compounds are derived from benzene (C_6H_6), at least conceptually and often in terms of their production. The phenyl group is hydrophobic and tends to resist oxidation and reduction.

Phenyl groups (like all aromatic compounds) have enhanced stability in comparison to equivalent bonding in aliphatic (non-aromatic) groups. This increased stability is due to the unique properties of aromatic molecular orbitals (Virtual Textbook, 2012).

Phenyl groups are found in many organic compounds, both natural and synthetic. Most common among natural products is the amino acid phenylalanine, which contains a phenyl group. A major product of the petrochemical industry is "BTX" consisting of benzene, toluene, and xylene - all of which are building blocks for phenyl compounds. One of the simplest phenyl-containing compounds is phenol, C_6H_5OH . It is often said the resonance stability of phenol makes it a stronger acid than that of aliphatic alcohols such as ethanol ($pK_a = 10$ vs. 16–18). As described above, benzyl and phenyl compounds are two forms of aromatic compounds and are, usually, present in the environment.

Aromatic compounds are inhibitors of methane biosynthesis in anaerobic treatment of solid wastes and industrial effluents. Anaerobic treatment of solid wastes and industrial effluents may be limited by the methanogenic bacteria inhibition exerted by these types of compounds, the production of biogas is not possible and the organic matter contained in the effluent is not reduced. These effluents poured in the nature can be the basis of the pollution. Benzyl and phenyl compounds, as the aromatic compounds, can be biodegraded in anaerobic digestion process but they can also be toxic to methanogenic bacteria (Field and Sierra, 1990; Lettinga and Hulshoff Pol, 1990; Chen et al., 2008).

The present paper aims to evaluate the inhibitors effects of benzyl and phenyl compounds in anaerobic digestion process and compare their inhibition on methane biosynthesis.

2. MATERIAL AND METHODS

2.1. Biomass

Pig manure from DAIPN farm of N'sele /Kinshasa (DR Congo) was digested in laboratory scale digester during about five months. The digested pig manure (sludge) was utilized as inoculums in our anaerobic toxicity tests. The digested pig 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 g COD-CH₄/g VSS .d (27±1 °C).

2.2. Stock solutions

2.2.1. Stock substrate solution: The stock solution of the substrate is a volatile fatty acid solution (VFA) composed of acetic acid neutralized to pH = 7 with NaOH solution. It is at the concentration of 100 g COD-CH₃COOH /l (chemical oxygen demand per liter);

2.2.2. Stock solution 1: macro-nutrients : NH₄Cl (170g/l); KH₂PO₄ (37 g/l); CaCl₂. 2H₂O (10 g/l); MgSO₄.4H₂O (37 g/l);

2.2.3.Stock solution 2: trace elements : FeCl₃.4H₂O (2000mg/l); CoCl₂.6H₂O (2000 mg/l); MnCl₂.4H₂O (500 mg/l); CuCl₂ (50 mg/l); H₃BO₃ (50 mg/l); (NH₄)₆Mo₇O₂.4H₂O (90 mg/l); Na₂SeO₃. 5 H₂O (100 mg/l) ; NiCl₂. 6 H₂O (50 mg/l mg/l) ; EDTA (1000 mg/l) ; HCl 36% (1 mg/l) ; yeast extract(200 mg/l) resazurin (500 mg/l);

2.2.4.Stock solution 3: Sulfide Na₂S (100 g/l) (; Field and Sierra,1990; Mambanzulua et al,1999; Mbuyu and Kayembe, 2000a).

2.3. Aromatic compounds

The used benzyl and phenyl compounds included: α-chlorotoluene (benzyl chloride), α-Bromotoluene (benzyl bromide), benzyl alcohol, chlorobenzene (phenyl chloride), bromobenzene (phenyl bromide), and phenol. Otherwise benzene and toluene were utilized as reference aromatic compounds. All aromatic compounds were of high purity available. The figure 1 shows benzyl and phenyl groups formulas.



Fig. 1: The structure of the benzyl group (A) and phenyl group (B) attached to an "R" group.

2.4. Anaerobic toxicity assay

Specific acetoclastic methanogenic activity measurements were performed with 1l glass serum bottles sealed with butyl rubber septa. Two ml stock solution 1; 1 ml stock solution 2, 2 drops stock solution 3 and 40 ml stock substrate solution were added to each serum bottle from the stock sludge. The serum bottle was filled to 1000 ml with oxygen free tap water which is flushed with nitrogen gas for at least 15 minutes. The flask were sealed with rubber septum cap and placed in a reciprocating shaker at 27±1 °C (room temperature).

The required quantity of inhibitory compound was added to each flask to provide the toxic concentration to be investigated. No toxicant was added to the controls. The toxicant concentrations had chosen as to cause an inhibition of the acetoclastic methanogenic activity ranging from 0-100 % (Sierra and Lettinga, 1989; Field and Sierra, 1990; Mpiana, 2010).

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 "IC₅₀". 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 (Field and Sierra,1990; Mambanzulua et al, 1999; Kalyuzhnyi et al.,2000;).

2.5. Methane gas measurement

The methane gas volume produced was measured by serum bottle liquid displacement systems (Mariotte flask system) as previously described (Field and Sierra, 1990; Mambanzulua et al, 1999; Mbuyu and Kayembe 2000b).

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 (Field and Sierra, 1990).

3. RESULTS AND DISCUSSION

3.1. Inhibition of specific methanogenic activity.

All concentrations of aromatic compounds examined exerted an inhibitory effect on the specific acetoclastic methanogenic activity.

Figure 2 shows the decrease in specific methanogenic activity with the increasing of the concentration of chlorobenzene. The IC₅₀ is calculated as the concentration of chlorobenzene corresponding to 50% of inhibition.

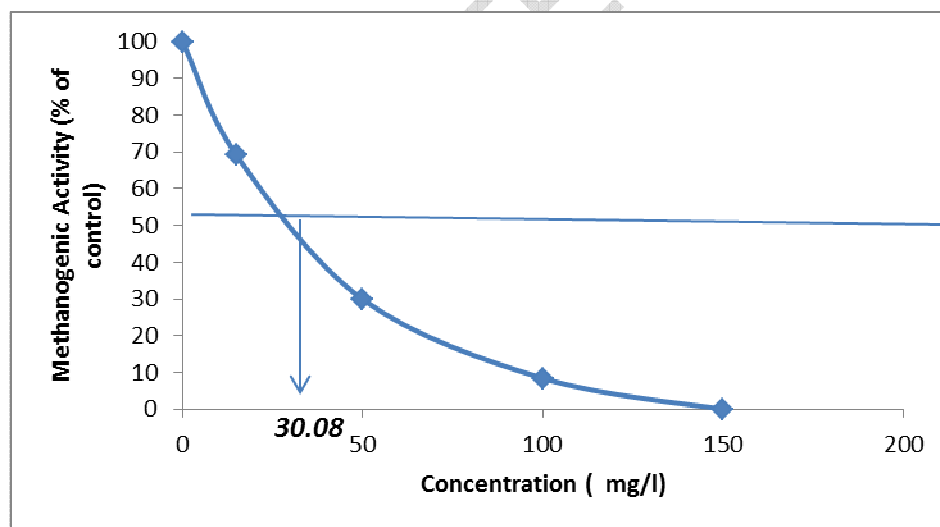


Fig 2: Methanogenic activity of digested pig manure exposed to chlorobenzene versus chlorobenzene concentration.

3.2. Methanogenic toxicity of benzyl and phenyl compounds

The inhibitory effect of benzyl and phenyl compounds: α -chlorotoluene (benzyl chloride), α -Bromotoluene (benzyl bromide), benzyl alcohol, chlorobenzene (phenyl chloride),

bromobenzene (phenyl bromide) , phenol and reference compounds (benzene, toluene) on the activity of acetoclastic methanogenic bacteria was studied at various levels, from concentrations that were nontoxic to those that were completely inhibitory concentration to acetoclastic methanogenic activity, as typified by the experiment with chlorobenzene in Figure 2. Table 1 summarizes the 50% inhibiting concentrations of monomeric tannin compounds evaluated in this study, ranked in decreasing order of toxicity.

Table 1: The IC₅₀ values observed in the study for the benzyl (Bn) and phenyl (Ph) compounds and reference aromatics.

N ^o	Compounds	IC ₅₀ (mg/l)	logP _{oct}
1	α-Chlorotoluene (BnCl)	0.61±0.03	2.30
2	Bromobenzene (PhBr)	2.90±0.16	2.99
3	α-Bromotoluene (BnBr)	8.67±0.24	2.92
4	Chlorobenzene (PhCl)	30.08±2.01	2.84
5	Toluene (BnH)	146.65±6.28	2.73
6	Benzene (PhH)	208.78±6.32	2.13
7	Phenol (PhOH)	1248.90±29.29	1.47
8	Benzyl alcohol (BnOH)	2391.37±28.67	1.10

Variation of the methanogenic toxicity as exhibited by the benzyl and phenyl compounds is illustrated in Figure 3.

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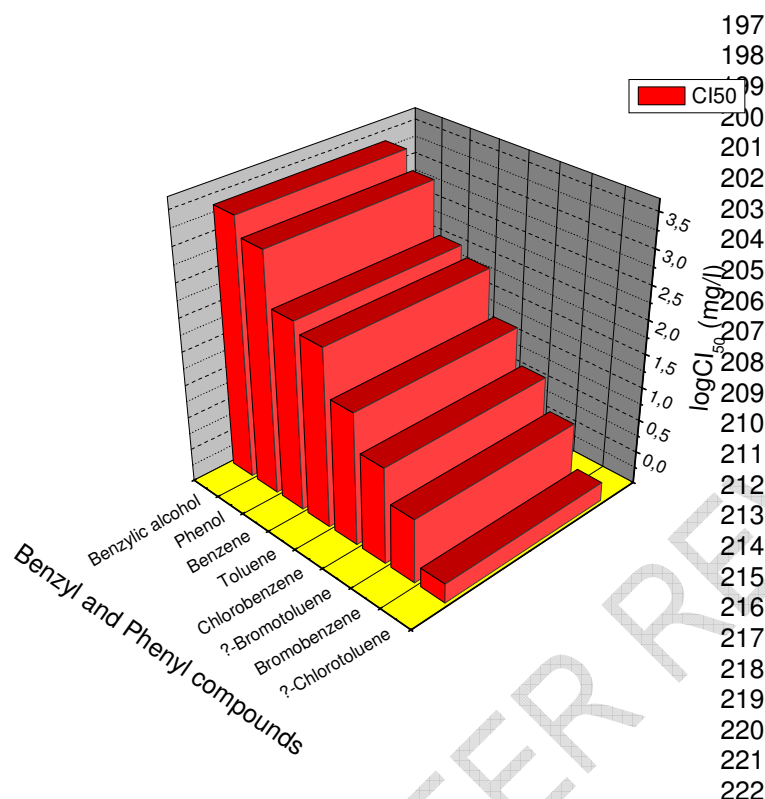


Fig. 3: Variation of the methanogenic toxicity as exhibited by benzyl and phenyl compounds.

The obtained results indicate that some general relationships exist between the nature of benzyl and phenyl compounds and their inhibitory effects on biosynthesis of methane by methanogenic bacteria. According to the figure 3, α -chlorotoluene and bromobenzene with 0.61 and 2.90 mg/l IC_{50} values, respectively, are the most toxic, while phenol and benzyl alcohol with IC_{50} values of 1248.90 and 2391.37 are less toxic.

The obtained results are comparable to those reported in our previous work (Kayembe et al, unpublished data) and by SIERRA and LETTINGA, 1989 for monosubstituted aromatic compounds. The addition of a functional group containing an oxygen or sulfur heteroatom to benzene, our reference compound, decreased the benzyl and phenyl compounds toxicity as in the case of OH substitution. However, the substitution of benzene H by Cl, Br, and CH_3 was associated with an increase in benzyl and phenyl compounds toxicity. This sufficiently demonstrates that the grafting of hydrophobic or hydrophilic substituent on the benzene, make the obtained compound more or less toxic (Debord, 2012)

3.3. Comparison of benzyl and phenyl methanogenic toxicity on methanogenic bacteria.

The methanogenic toxicity of benzyl and phenyl compounds with the same substituents is compared in the figure 4. About our references compound, the benzene is a phenyl

compound with H as the substituent while the toluene is a benzyl compound with, also, H as the substituent.

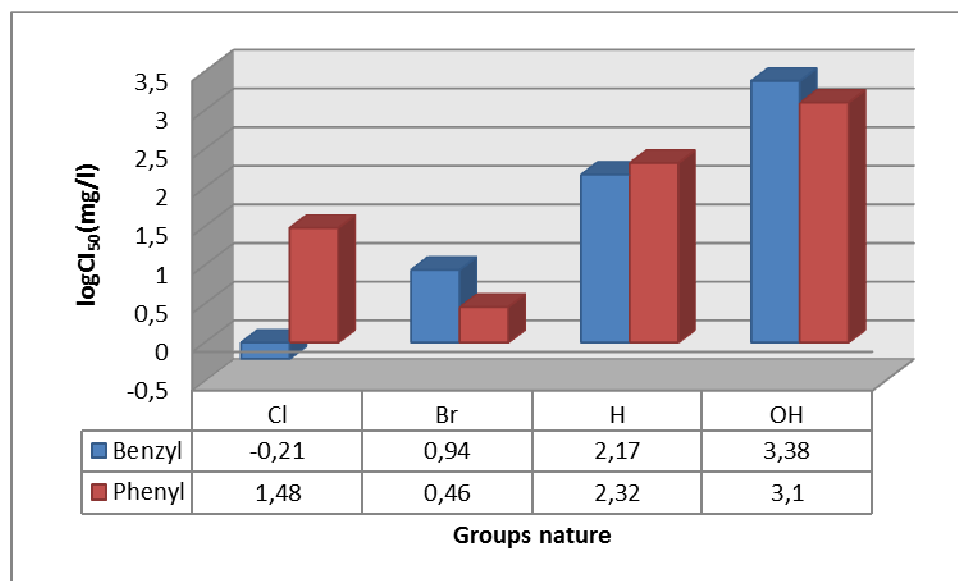


Fig. 4: Methanogenic toxicity of benzyl compounds versus phenyl one.

According to the figure 4, the toluene is more toxic than benzene. Comparing the phenyl and benzyl compounds with the same substituents, the benzyl chloride is more toxic than phenyl chloride while benzyl bromide is less toxic than phenyl bromide. Otherwise, benzyl alcohol is less toxic than phenol.

These results are comparable to those obtained by Sierra and Lettinga (1989) for benzyl alcohol and phenol; toluene and benzene. But the others couples of benzyl and phenyl compounds were not studied by these authors.

3.4. Correlation of the methanogenic toxicity with aromatic compounds hydrophobicity.

Correlations between toxicity and partition coefficient within series of organic contaminants structurally related have been reported by a number of research groups using fish, ciliate or microorganisms as tests organisms. Therefore, when comparing compounds that possess different types of substitutions, a perfect correlation with logP_{oct} of the compound cannot be expected. A higher correlation could potentially be obtained by comparing compounds in homologues series (Sierra and Lettinga, 1989; Donlon, 1995).

To determine if the lipophilic character of benzyl and phenyl compounds tested could be correlated with their methanogenic toxicity, the logarithm of the IC₅₀ values of these aromatics were plotted against the logarithm of the octanol-water partition coefficient (logP_{oct}) of the aromatic compounds. Figure 5 shows the correlation line between the methanogenic toxicity and partition coefficient logP_{oct} for phenyl compounds (chlorobenzene, bromobenzene, benzene and phenol). No correlation was found for benzyl compounds (benzyl chloride, benzyl bromide, benzyl alcohol and toluene) methanogenic toxicity (IC₅₀) and partition coefficient (logP_{oct}) correlation.

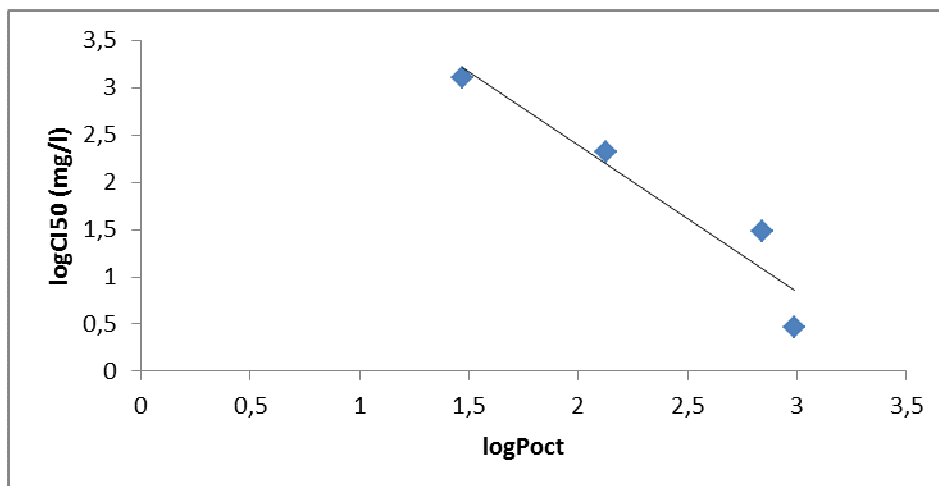


Fig. 5: Effect of hydrophobicity on phenyl methanogenic toxicity: methanogenic toxicity (IC₅₀) and partition coefficient (logPoct) correlation ($R^2 = 0.911$).

It can be notice that there is a significant correlation between the toxicity of phenyl compounds and their hydrophobicity ($R^2 = 0.911$). This causes that the more hydrophobic molecule is, the more readily it crosses the cell membrane and becomes highly toxic and inversely (Debord, 2012; Mpiana, 2010).

4. CONCLUSION

The obtained results indicate that general relationships exist between the nature of benzyl and phenyl compounds and their inhibitory effects on biosynthesis of methane by methanogenic bacteria. According to these results, α -chlorotoluene and bromobenzene with 0.61 and 2.90 mg/l IC₅₀ values, respectively, are the most toxic compounds, while phenol and benzyl alcohol with IC₅₀ values of 1248.90 and 2391.37 are less toxic.

Comparing the phenyl and benzyl compounds with the same substituents, the benzyl chloride is more toxic than phenyl chloride while benzyl bromide is less toxic than phenyl bromide. Otherwise, benzyl alcohol is less toxic than phenol.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

K. Kayembe designed the study and performed the experimental work, L. Basosila performed the statistical analysis, wrote the protocol; P.T.Mpiana 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.

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