

Prevalence and survival of drug-resistance pathogens in anaerobic digestion treatment of cattle manure

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HIGHLIGHTS

High percentage of *Escherichia coli* was persistent after anaerobic digestion of cattle manure.

Strains showed resistance to ampicillin, ampicillin-sulbactam, and chloramphenicol.

Present study suggested discussion on the use of antimicrobials in farms, and necessity to improve of anaerobic digestion process to reduce pathogens.

Keywords

Anaerobic digestion; Dairy cattle manure; Fertiliser; Resistance pathogens

INTRODUCTION

In Brazil, rapid growth of dairy industry has led to difficulties in the management of cattle manure. Biowaste is known to contain pathogenic enteric microorganisms that may some risks to human and animal health. In addition, widespread use of antibiotics in the cattle farm increases the presence of drug-resistant bacteria and their subsequent release to the environmental. Anaerobic digestion is an alternative way to handle biowaste, producing biogas, and residues that can be used as fertilisers (Bagge et al., 2005; Holm-Nielsen et al., 2009). The objective of this work was to evaluate the microbiological safety of the anaerobic digestion effluents by analysing the occurrence of enterobacteria and its antimicrobial susceptibility in four continuous biodigesters. Dairy cattle manure was fed to the biodigesters and operated with hydraulic retention time of 31 days.

MATERIALS & METHODS

Influent and effluent samples from the biodigesters were collected at different times to analyse the biowaste by selective isolation of enterobacteria. Biochemical identification was performed using commercial kits. Antimicrobial susceptibilities were determined by agar-dilution method (CLSI, 2012), whereas the level of antibiotic resistance was determined by the calculation of the multiple antibiotic resistance index (MAR) (Krumperman, 1983). Besides bacterial counts in decimal dilutions ($10^{-1}/10^{-6}$) of the biowaste were made, and aliquots of 100 μ L were spread on Eosin-Methylene Blue Agar.

RESULTS AND DISCUSSION

The average load of enterobacteria was 4.4×10^8 CFU/ml for the influent and 1.4×10^5 CFU/ml for the biodigesters effluent. A total of 211 enterobacteria were obtained from influent (n=28) and from effluent (n=183). *Escherichia coli* was the most dominant species in both influent and effluent (82,1% and 79,8%, respectively), followed by *Enterobacter* sp., *Salmonella* sp., and *Serratia* sp. Antimicrobial drug susceptibility patterns for the isolated microorganisms in influent and effluent samples were showed in Table 1. Bacteria showed resistance to ampicillin, ampicillin-sulbactam, and chloramphenicol. High susceptibility rates were observed for meropenem, levofloxacin, ampicillin, and cefepime. 25,6% of bacteria presented a multiple antibiotic resistance index (MAR) > 0.2, ranging between 0.2 to 0.3. The high concentrations of *E.coli* in the biowaste and the

prevalence of antibiotic resistance suggested that digested residues produced could represent a significant source of antibiotic-resistant bacteria. It is known that antibiotic-resistant genes can be passed between diverse species of microorganisms and present a potential risk for human and animal health. On the whole, our findings suggested discussion on the use of antimicrobials in farms, because use of these drugs inevitably selects for resistance of both commensal and pathogenic micro-organisms exposed to the agents (Venglovsky et al., 2009).

CONCLUSIONS

The persistence of antimicrobial-resistant pathogens after the biodigestion may be a risk when using digested residues on arable land. Further studies are necessary to improve the anaerobic digestion process in order to ensure a technological (biogas production) and microbiological (reducing microorganisms) optimization.

Table 1. Drug susceptibility patterns of bacteria observed in the biowaste samples of the biodigesters influent and effluents. Susceptibility patterns - S: sensitivity, IR: intermediate resistance, R: resistance.

	Tested antimicrobial drugs	Minimum inhibitory concentrations ($\mu\text{g/mL}$)			Susceptibility patterns (%)		
		MIC _{50%}	MIC _{90%}	Range	S	IR	R
Influent	Meropenem	0,06	0,06	0,06 - 1	100	-	-
	Gentamicin	1	64	0,25 - 128	82	-	18
	Amikacin	2	4	1 - 32	96	4	-
	Levofloxacin	0,06	0,24	0,06 - 1	100	-	-
	Sulphazotrim	0,06	76	0,03 - 76	82	-	18
	Cefepime	0,06	0,12	0,06 - 1	100	-	-
	Chloramphenicol	4	8	4 - 32	96	-	4
	Ampicillin-sulbactam	4	64	1 - 128	79	7	14
	Ampicillin	8	32	2 - 512	64	14	21
Effluent	Meropenem	0,06	0,25	0,06 - 2	99,4	0,5	-
	Gentamicin	1	1	0,06 - 128	99,4	-	0,5
	Amikacin	4	4	0,25 - 32	99,4	0,5	-
	Levofloxacin	0,06	0,12	0,06 - 1	100	-	-
	Sulphazotrim	0,06	4,8	0,06 - 8	100	-	-
	Cefepime	0,06	0,12	0,06 - 8	100	-	-
	Chloramphenicol	4	8	0,06 - 32	89	9	3
	Ampicillin-sulbactam	8	128	1 - 512	76	8	16
	Ampicillin	8	64	0,06 - 1024	64	19	17

ACKNOWLEDGEMENTS

This work was supported by: CNPq, FAPEMIG, CAPES.

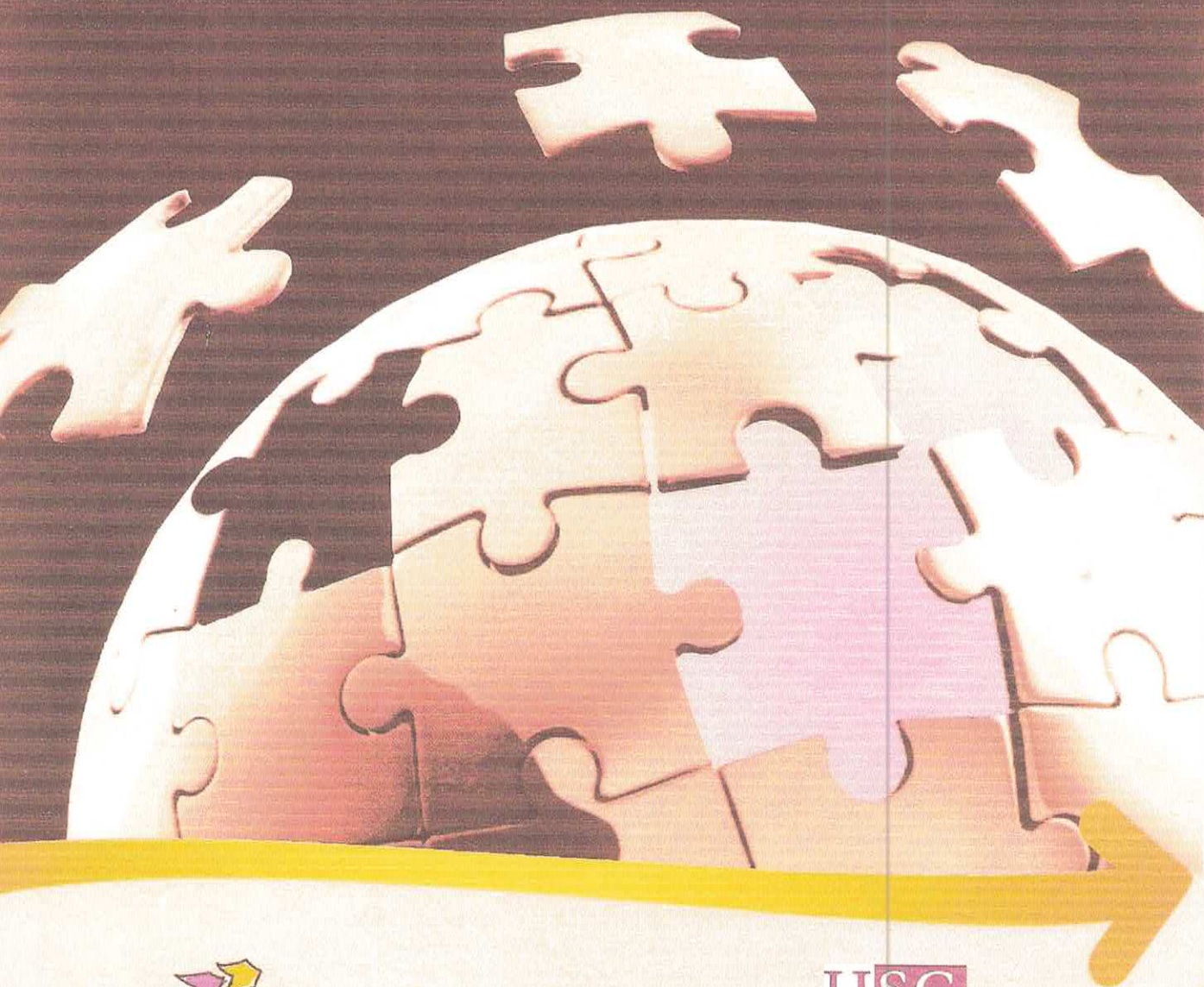
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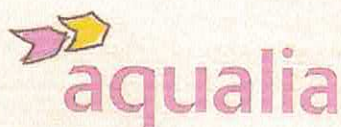
Proceedings of 13th World Congress on Anaerobic Digestion

25th-28th June 2013

Santiago de Compostela (Spain)



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