EU-India STI Cooperation Days
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Madalena Alves[1], Farhana Masood[2], Abdul Malik[2]

1- University of Minho, Portugal

2- Aligarh Muslim University India

This event is co-financed by the European Commission through the involvement of several projects.

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Hosted by National Geophysical Research Institute (NGRI- CSIR).

Biological Treatment (anaerobic/aerobic) of Pulp and Paper Industry Wastewater with Special Reference to Genotoxicity of Waste Before and After Treatment

Abdul Malik
Aligarh Muslim University

Madalena Alves
University of Minho

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DEPARTMENT OF AGRICULTURAL MICROBIOLOGY

➢The department has its mandate as to create a centre of academic excellence in the field of education and research in Agricultural microbiology and newer Microbial technology; provides a sound academic background and environment for an overall development of personality for a successful career in Microbiology which fosters continuous improvement and innovation in the subject.

➢Keeping in view the national demands, the Department of Agricultural Microbiology, Faculty of Agricultural Sciences, A.M.U., is imparting teaching and training at Post-graduate and Doctoral levels on various aspects of Microbiology such as, Soil Microbial Ecology, Immunology, Biochemistry, Food Microbiology, Environmental Microbiology, Industrial Microbiology and Agricultural Biotechnology and Molecular Microbiology. Indeed, teaching and hand-on training in such areas have a potential for creating opportunities/job prospects for the Post Graduate/ Ph. D students in the Biotechnology industry, Agriculture sectors and national/International Institutes and Universities. Keeping in view of the significant demand for trained Microbiologist, the Department of Agricultural Microbiology, is striving hard to bring excellence to the existing program of PG teaching & Research.

➢In fact, the country's prosperity is inseparably linked to agricultural progress, and a key to achieving this progress is a shift from resource-dependent agriculture to a science-based agriculture., The Agricultural microbiology and applied microbial technology has emerged as an important discipline, offering solutions to multifarious problems, presently encountered in developing sustainable agriculture and increasing crop productivity and food security environmental and human health protection and climate change.

➢The faculty members have excellent records of publication in journals with high impact factors and received fair citation in the scientific community. The department has completed various extramural research projects funded by national (UGC, DST, DBT, UPCST etc.) and international (Germany & Portugal) funding organization.

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Biological Treatment anaerobic/aerobic) of Pulp and Paper Industry Wastewater with Special Reference to Genotoxicity of Waste Before and After Treatment

Indo-Portuguese cooperation Project

Exchanges between Aligarh and Braga

Madalena Alves and Diana Sousa visited Aligarh between 11 and 18 March 2011

Farhana Masood visited Braga between 1st and 30th April 2011

Abdul Malik visited Braga between 15 November and 15 December 2011

Raquel Pereira visited Aligarh between 19 March and 17 April 2012

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Common research work with pulp and paper wastewater samples collected in India and Portugal

Outputs

1 Book Chapter of Minho team in a book edited by Abdul Malik

Pereira, Luciana; Alves, M.M. **Dyes-Environmental Impact and Remediation. In: Environmental Protection Strategies for Sustainable Development**, Abdul Malik, Elisabeth Grohmann (Eds), pp: 111-162, (ISBN: 978-94-007-1590-5), Springer, Dordrecht, 2012

1 Paper under preparation

1 book edited

Management of Microbial resources in the Environment. Abdul Malik, Elisabeth Grohmann, Madalena Alves (Eds) ISBN 978-94-007-5930-5, Springer.

Extended collaboration

Two FP7 applications

(> threshold but unfortunatly not funded)

CONTAMINATED SITES UNDER INVESTIGATION

Paper and Pulp Industry Wastewater

Portuguese Effluent Sample

- high lignin content
- high absorbable organic halide (AOX) concentration
- color,
- low biodegradability (COD/BOD) ratios
- Potential toxicity problems

- Paper industry is one of the biggest user of water.
- More than 47,000–80,000 gallons of wastewater discharged during paper production.
- About 500 organochlorines have been identified eg. chloroform, chlorate, resin acids, chlorinated hydrocarbons, phenols, catechols, guaiacols, furans, dioxins, syringols, vanillins, etc. collectively termed as‘‘adsorbable organic halides’’ (AOX).
- AOX are biologically persistent, recalcitrant and highly toxic .
- Their toxicity range from carcinogenicity, mutagenicity to acute and chronic toxicity.

OBJECTIVES

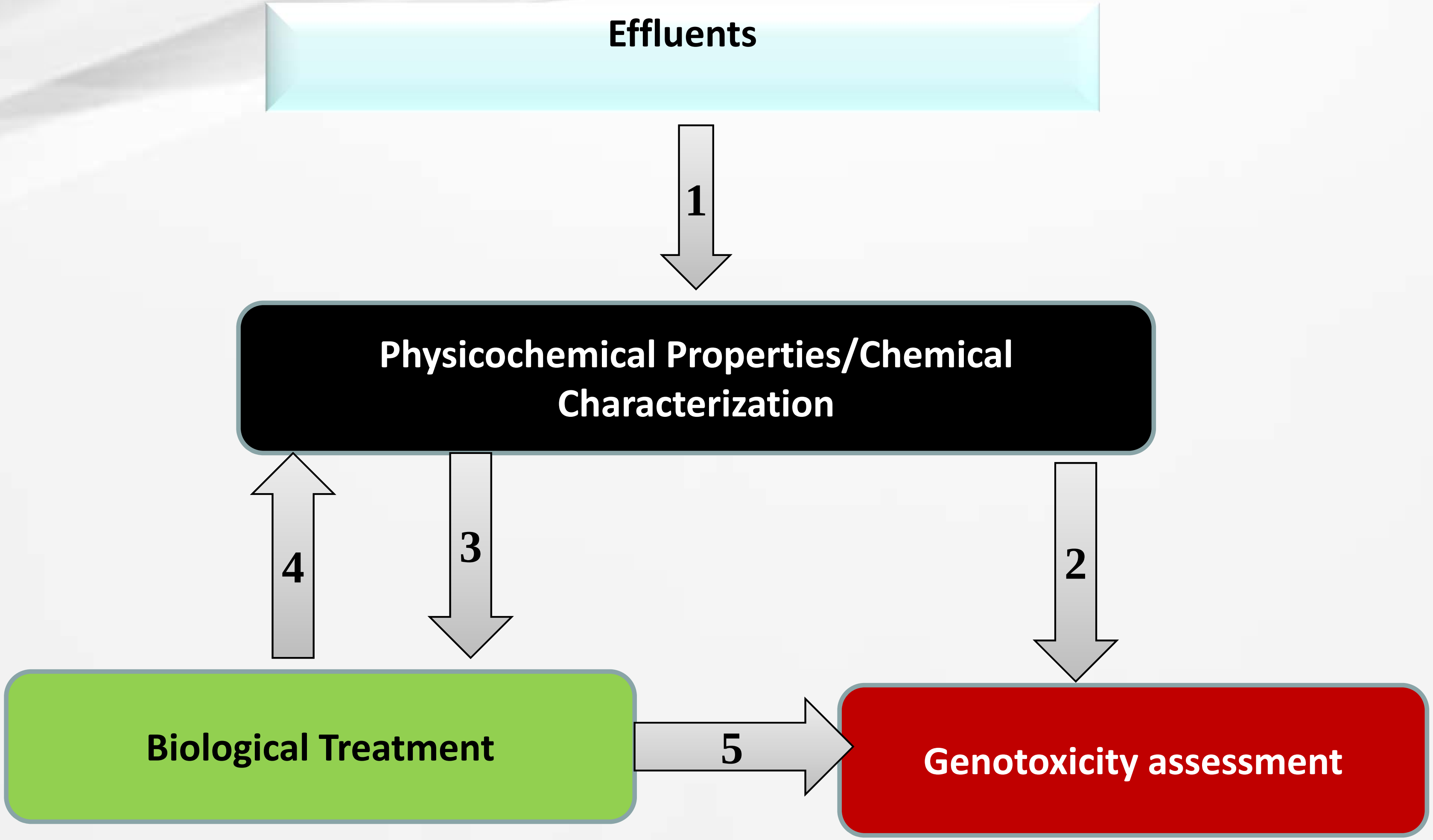
Effluents
1
Physicochemical Properties/Chemical Characterization
4
3
2
Biological Treatment
5
Genotoxicity assessment

Table 1. Physico-chemical characteristics of the wastewaters before biodegradation

	Indian Wastewater	Portuguese Wastewater
pH	4.0	6.87
COD	7300	225
Ammonium	7.62	< 1
Nitrate	8.62	1.46
Phosphate	64.7	2.20
Total Nitrogen	76.40	< 20
Total Phenolics	34.59	3.03

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Table 2. Physico-chemical characteristics of the wastewaters after anaerobic biodegradation

	Indian Wastewater	Portuguese Wastewater
pH	7.3	7.37
COD	443	170
Ammonium	2.38	1.70
Nitrate	< 1	< 1
Phosphate	23.1	25.40
Total Nitrogen	< 5	< 5

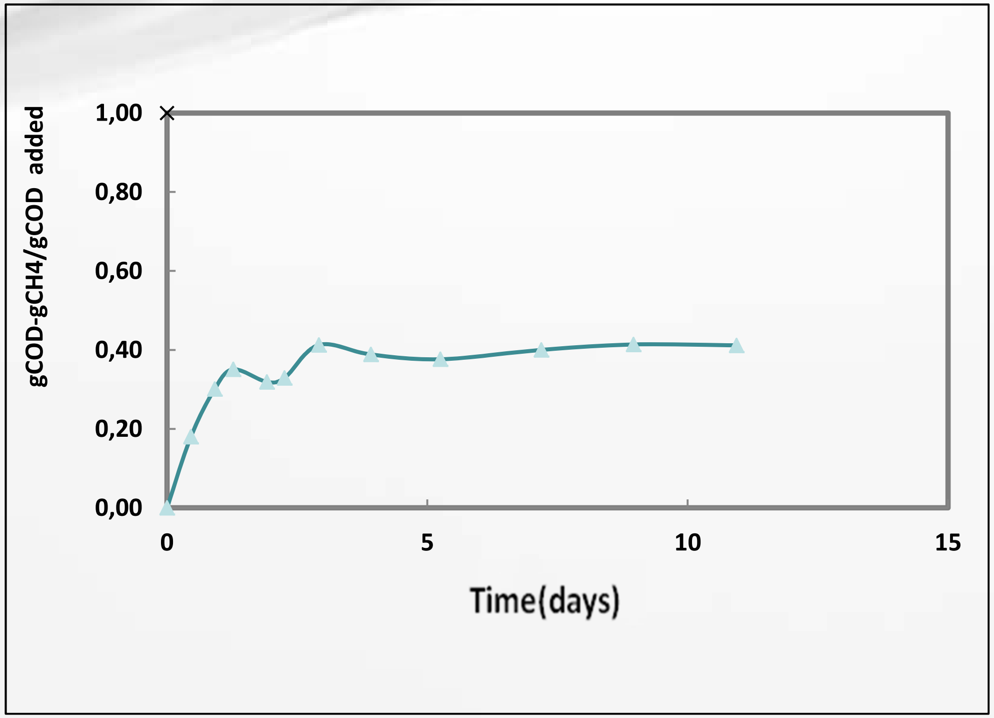

Figure. Methane production in the biodegradability assay conducted with Indian wastewater

Methodology

Composition of Indian and portuguese wastewaters were characterized before and after biodegradation in terms of:

- **Hexane extract**
- **Choloform extract**
- **Acidic DCM fraction**
- **Basic DCM fraction**

All the extracts were analysed by GC-MS

Example of compounds identified

Hexane Extract

Indian Wastewater	Portuguese Wastewater
Cyclopropaneoctanoic acid 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester Bis (2-ethylhexyl) phthalate Diphenylether 1,9-Nonanediol Pyrido[2,3-d] pyrimidine-2, 4 (1H, 3H	Cyclotetrasilonaxe, octamethyl Acetic acid, N(1,3)-hydroxy-1-phenylethylhydrazide Oxime-methoxy-phenyl 4-hydroxymandelic acid, ethylester-di-TMS Cyclotetrasilaxane, octamethyl Cyclotrissilaxane, hexamethyl Heptasilaxane,n-tetradecanemethyl Octasilaxane,n-hexadecamethyl

All the extracts were analysed (next slides)

Table 3. Compounds identified in Indian wastewater using GC-MS

Sample	Compounds identified
Hexane extract)	Cyclopropaneoctanoic acid
	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester
	Bis (2-ethylhexyl) phthalate
	Diphenylether
	1,9-Nonanediol
	Pyrido[2,3-d] pyrimidine-2, 4 (1H, 3H)
Chloroform extract	Phenol, 2, 2methylene,6,1,1-dimethylethyl-4ethyl
	Bis (2-ethylhexyl) phthalate
	Ethyl N-propyl disulphide
	Heneicosane
	1-Decanol,2,hexyl
	1-hexadecanol,2-methyl
	Tetratetracontane
	Heneicosane
	Tetrapentacontane,1,5,4-dibromo
Acidic DCM fraction	Hexadecen-1-ol, trans-9-
	Dotriaconatne
	9-methylnonadecane
	1-Octadecanol
	Di-n-octyl phthalate
	Tetracosane
Basic DCM fraction	Phenol, 2,4- bis (1,1-dimethylethyl)-
	Di-n-octyl phthalate
	Phenyl N-methylcarbamate
	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester
	Nonadecane

Table 4. Compounds identified in Portuguese wastewaters using GC-MS

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Sample	Compounds identified
Hexane extract	Silanediol, dimethyl
	Cyclotetrasilonaxe, octamethyl
	Acetic acid, N(1,3)-hydroxy-1-phenylethylhydrazide
	Oxime-mthoxy-phenyl
	4-hydroxymandelic acid, ethylester-di-TMS
	Cyclotetrasilaxane, octamethyl
	Cyclotrissilaxane, hexamethyl
	Cyclotetrasilaxane, octamethyl
	Heptasilaxane,n-tetradecanemethyl
	Octasilaxane,n-hexadecamethyl
Chloroform extract	1,3-Diazine
	1,2-Benzenedicarboxyl acid, bis82-methyl propyl ester
	Cycloaaactasiloxane, hexadeamethyl
	2,3-Diphenylcyclopropylmethylphenylsulfoxide, trans
	Benzene-1,1-(2-methyl-2-(phenylthio) cyclopropyldenel bis
Basic DCM fraction	Borneol
	3-Cyclohexene-1-methanol,n,n,4-trimethyl
	Cyclooctasiloxane, hexadecamethyl
	Phenol,2,5,bis (1,1dimethylethyl)
	Z-8-Methyl-9-teetradecenoic acid
	Chlolestan-3-ol,2-methylene
	Phathalic acid, isobutyloactylester
Acidic DCM fraction	Syn-Tricycloact-5-ene-335688-hexamethyl
	B-Vatirenene
	Spirolcyclopropane-1,1,naphthalen-4-one
	Trycyclo(3,1) hexane , 3,6-diethyl-3,6-dimethyl
	1-hexadecanol
	Naphthalene-dimethyl
	Phenol 2,4-bis(1,1-dimethylethyl)
	2-Butyloxycarbonyloxy-1,1,10-trimethyl-6,9-epidioxydecain
	Z-8-Methyl-9-teetradecenoic acid
	Phthalic acid, isobutylactylester
	Propanoic acid
	tert-Hexadecanethiol
	Tetradecane,2,6,10-trimethyl

Table 5. Compounds identified in Indian wastewater after biodegradability test using GC-MS

Sample	Compounds identified
Hexane extract	Syn-Tricycloact-5-ene-335688-hexamethyl
	Isolangifolene-9,10 dehydro
	Diphenylether
	Tran-2,2-Bisabolene epoxide
	1-Hexadecanol
	1,5,9-Cyclodecatriene-1,5,9-Thrimethyl
	Z-(1,3,4-Epoxy) Tetradec-11-en-1-oleacetate
	Cyclopropaneoctanoic acid
	Propanoic acid
Chloroform extract	Phenol, 3-methyl
	Phenol, 4-methyl
	7-Tetracyclo-undecane-4,4,11,11-tetramethyl
	Diphenylether
	1-Docasene
	1,5,9-Cyclododecatriene,1,5,9-trimethyl
	3-Chloropropionic acid, heptadecylester
	Ethanol, 2-(octadecyloxy)
	1-Decanol,2,hexyl
	1-hexadecanol,2-methyl
	(2,3-Diphenylcyclopropylmethylphenylsulfoxide, trans
	Tetrapentacontane,1,5,4-dibromo
	Phenol, 2, 2methylene,6,1,1-dimethylethyl-4ethyl
	Benzene-1,1-(2-methyl-2-(phenylthio) cyclopropyldenel bis
Basic DCM fraction	Hexane,3,4,4,4-tetrafluoro
	Phenol, 4-methyl
	Phenol,2-ethyl
	1-Nonedecane
	2-propenoic acid, pentadecylester
Acidic DCM fraction	Dibutyl phtalate
	Ciclohexanecarboxylic acid
	Phenol, 2-ethyl
	Diphenylether
	3-Eicosane
	Phenol 2,4-bis(1,1-dimethylethyl)
	12-Methyl-EE-2.13.octadecain-1-ol
	Propionic acid,3,mercapto-dodecylester

Table 6. Compounds identified in Portuguese wastewaters after biodegradability test using GC-MS

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Sample	Compounds identified
Hexane extract	Isolangifolene-9,10 dehydro
	1-Docasene
	Trans-2-Hexadecenoic acid
	1,2-Benzenedicarboxyl acid, butyl2-ethylhexylester
	1-Decanol,2,hexyl
Chloroform extract	Ethane 1,1achloro
	1-docasene
	trans-2-Hexadecenoic acid
Basic DCM fraction	3-Benzylsulfonyl-2,6,6-trimethyloic acid-3,1,1 heptane
	3-Eicosene
	3-Cloropropionic acid, heptadecylester
	Lenthiorine
Acidic DCM fraction	Z-1-Chloro-2-methylsulfonylethylene
	1,2.3-Tritriolane
	(2,2,6-Trimethyl-bicyclo hept-1yl-methanol
	Z-8-Methyl-9-teetradecenoic acid
	Phthalic acid, isobutylactylester
	propanoic acid didodecyclester

Reversion of *Salmonella* tester strains (TA97a, TA98, TA100, TA102 and TA104) was analyzed for all extracts (next slide – one example for hexane and chloroform extracts)

Table 7. Reversion of *Salmonella* tester strains in the presence of hexane extracted Indian wastewater sample

		Doses (µl/plate)						
Strain	Control	1	5	10	20	40	Mi	m
TA97a	89 ± 6	181 ± 10 (2.0)	228 ± 9 (2.5)	284 ± 11 (3.1)	312 ± 8 (3.5)	276 ± 8 (3.1)	0.91	3.5
TA98	29 ± 4	119 ± 7 (4.1)	167 ± 8 (5.7)	241 ± 6 (8.3)	377 ± 12 (13.0)	319 ± 10 (11.0)	2.48	6.7
TA100	124 ± 7	208 ± 8 (1.6)	260 ± 11 (2.0)	316 ± 8 (2.5)	390 ± 11 (3.1)	338 ± 13 (2.7)	0.76	4.5
TA102	239 ± 10	289 ± 13 (1.2)	331 ± 10 (1.3)	400 ± 9 (1.6)	468 ± 13 (1.9)	411 ± 12 (1.7)	-0.04	4.0
TA104	328 ± 12	371 ± 14 (1.1)	427 ± 13 (1.3)	478 ± 17 (1.4)	523 ± 14 (1.5)	485 ± 14 (1.4)	-0.52	3.5

Table 8. Reversion of *Salmonella* tester strains in the presence of chloroform extracted Indian wastewater sample

		Doses (µl/plate)						
Strain	Control	1	5	10	20	40	Mi	m
TA97a	82 ± 6	138 ± 6 (1.6)	162 ± 8 (1.9)	221 ± 9 (2.6)	275 ± 9 (3.3)	241 ± 9 (2.9)	0.85	3.7
TA98	35 ± 5	147 ± 8 (4.2)	200 ± 7 (5.7)	267 ± 10 (7.6)	312 ± 14 (8.9)	278 ± 11 (7.9)	2.10	4.8
TA100	130 ± 9	178 ± 11 (1.3)	243 ± 11 (1.8)	330 ± 8 (2.5)	371 ± 12 (2.8)	339 ± 10 (2.6)	0.61	4.7
TA102	241 ± 10	283 ± 13 (1.1)	325 ± 9 (1.3)	386 ± 11 (1.6)	439 ± 13 (1.8)	394 ± 13 (1.6)	-0.19	3.6
TA104	325 ± 11	359 ± 1 (1.1)	418 ± 12 (1.2)	472 ± 16 (1.4)	516 ± 16 (1.5)	478 ± 16 (1.4)	-0.53	2.9

CONCLUSIONS

- Both Indian and Portuguese wastewater samples showed considerable biodegradation
- All the wastewater samples are heavily contaminated with various organic compounds
- Genotoxicity of different wastewater samples arranged as follows: dichloromethane extracted water samples > hexane extracted water samples > chloroform extracted water samples.
- TA98 was found to be the most responsive strain in terms of mutagenic index, mutagenic potential and induction factor, indicating the presence of frame shift mutagens in the test samples.
- Wastewater from India has higher mutagenic index, induction factor, and more slope (m) of the response with increasing doses in comparison to wastewater from Portugal.


Thank you!