
EXPLORATION OF MICROBIAL CONSORTIUM FOR THE DEGRADATION OF RECALCITRANT PHARMACEUTICAL COMPOUNDS FROM SOIL & PHARMACEUTICAL EFFLUENT

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Abstract: Xenobiotics, such as pesticides and pharmaceuticals are the most frequently found pollutants in soil and water ecosystems, because they are used widely and in large quantities. To remove these contaminants microbial bioremediation is found to be a ecofriendly and cost effective means. Some of the Polycyclic Aromatic Hydrocarbons (PAHs) are recalcitrant, potential carcinogens & can bioaccumulate in food chain. Many polycyclic aromatic hydrocarbon (PAH) degrading microorganism including bacteria, fungi and actinomycetes exhibits broad biodegradative profile either in single or in consortium. Thus isolates, of anthracene and naphthalene degrading microorganism from soil and pharmaceutical effluent samples, either in single or in consortium might be a better option for carrying out bioremediation of soil and wastewater or pharmaceutical chemical contaminated water.

In this project 14 anthracene (6 bacteria & 8 fungi) and 17 naphthalene degraders (6 bacteria & 11 fungi) were isolated from soil & pharmaceutical effluent samples. The concentration of anthracene & naphthalene used was 1 µg/ml in selective medium for isolation. Biodegradation of 100 µg/ml of Ciprofloxacin, Diclofenac, Ibuprofen & Ranitidine [used as a single substrate /co-substrate] was carried out. After 15 days of incubation with potent anthracene and naphthalene degraders it was found that not a single bacterial or fungal cultures, but a consortia of bacteria, consortia of fungi and consortia of bacteria & fungi work more efficiently. In this work we aim to search for new highly active microbial consortia. Our work highlights the qualitative study of exploration of microbial consortia which may support quantitative studies.

Keywords: Xenobiotics, Recalcitrant, pharmaceuticals, PAHs, co-substrate, biodegradation, microbial consortium.

Introduction: Anthracene and naphthalene are Polycyclic Aromatic Hydrocarbons (PAHs), which have fused ring. They are the pollutants in atmosphere and relatively resistant to biodegradation because of their hydrophobicity and hence pose serious problems to environment and living beings.[1]

The term “pharmaceuticals” refers to a complex class of compounds used worldwide in human and veterinary medicine. Environmental concern over the presence of recalcitrant active pharmaceutical compounds in the environment is increasing as they affect the microbial community (antibiotic resistance), and can be taken up by crops.[2] They produce water (pharma effluent) which are also known to be toxic to fishes and other marine animals.

Ciprofloxacin or 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-piperazinyl quinoline carboxylic acid, being active against many gram-negative and gram-positive pathogenic bacterial species. CIP and other FluoroQuinones were shown to be tightly bound by human feces and soil, being no longer bioavailable and hence, strong binding may delay biodegradation and could partly explain the supposed recalcitrance of FluoroQuinones.[3]

Diclofenac or 2-[(2, 6-Dichlorophenyl) amino] benzenoic acid and Ibuprofen or α-Methyl-4-[isobutyl] phenylacetic acid are anti-inflammatory drugs.[4,5] Acidic drugs, such as ibuprofen and diclofenac at levels of 0.05 µg/ml reduced diversity and caused shifts in the structure of microbial communities.[6]

Ranitidine or Zantac is a histamine H₂-receptor antagonist.[7] All the described drugs are reported to cause pollution.

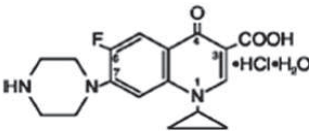
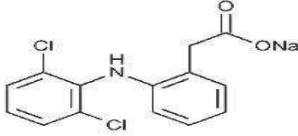
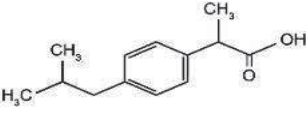
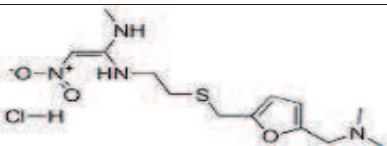
Pharmaceutical	Chemical structure
Ciprofloxacin	
Diclofenac	
Ibuprofen	
Ranitidine	

Figure: Chemical structure of pharmaceuticals.

Chemical, physical as well as biological methods are used for PAHs and pharmaceuticals degradation. Above all, biological methods are favored because of their good results and low costs. According to IUPAC definition 'Degradation is caused by enzymatic process resulting from the action of cells'.[4] Two main mechanism of biodegradation are possible. Co-metabolism takes place when a compound biodegrades only when other organic compounds are present and are acting as substrate for the microorganisms. The other mechanism is called catabolic metabolism and it takes place when the compound in itself can be the only source of substrate for the microorganisms.

Biodegradation is also highly dependent on the microbial communities.[6] It was reported that *Pseudomonas fluorescence* and *Bacillus megatherium* and fungi such as *Aspergillus species*, *Rhizopus species* and *Penicillium species* were proven to degrade benzene and toluene (pharmaceutical solvents)[8].

In Nigeria's research study, spent white-rot fungi white-rot fungus *Pleurotusostreatus* metabolizes 99% recalcitrant Carbamazepine (CBZ) [2]. And two strains of *Pleurotus* were degraded 17α-

Ethinylestradiol (EE₂) and CBZ individually [9].

Rhodococcus rhodochrous and *Pseudomonas aeruginosa* were shown to cometabolize SMX [10]. Many PAHs degrading microorganism including bacteria, fungi and actinomycetes, exhibit broad biodegradative profile either singly or in consortium. Thus, potent isolates of anthracene and naphthalene degrading microorganism (from soil and pharmaceutical effluent samples), either in single or in consortium might be a better option for carrying out bioremediation of contaminated soil and water.

Materials And Methods:

Sample collection:

Soil samples were collected from different sites at Kajgaon, Badlapur, Wada, Taloja, Wangni, Ambarnath, Pune, Thane, Kalyan & Karjat.

Pharmaceutical effluent samples were collected from Bhushal Laboratories, Badlapur. The effluent samples were collected in clean carboys of the capacity of one liter and stored in refrigerator

Anthracene and naphthalene were provided by Smt. CHM College itself, Ulhasnagar,

Maharashtra.

Enrichment:

Enrichment of all the samples was carried out by using Minimal Salt Medium (MSM) containing anthracene or naphthalene in 1 and 10 µg/ml concentration incubated at RT for 15 days.

Isolation:

For isolation of anthracene and naphthalene biodegraders Mineral Salts Medium supplemented with 1µg/ml of anthracene or naphthalene as a sole source of carbon was used. Incubated at RT for 15-16 days. For maintenance the isolated PAH degrading colonies was re-isolated on respective MSM slants and incubated at RT for 10-12 days.[11]

Identification:

Morphological, cultural & biochemical characteristics study of bacterial isolates was studied by gram staining, colony characteristics, various standard biochemical tests[11] viz; Lactose Fermentation test, IMViC test, Triple Sugar Iron Agar (TSI) test and Motility test for Oxidase Negative Gram negative rods (anthracene and naphthalene degraders).

Bacteria were identified by catalase, oxidase, carbohydrate fermentation, sodium (Na⁺) requirement for growth, IMViC and TSI biochemical tests for respective organisms.

Morphological study of fungal cultures was done by carrying out wet mount using 1% Lactophenol cotton blue.

Biodegradation studies:

All the pharmaceuticals were obtained from Thakur Medical stores, Kalyan. They were used in 10 µg/ml concentration in Mineral Salts Medium. Growth characteristics are correlated with biodegradation.

The present work was aimed to search for new highly active bacterial and fungal isolates, with a broader and more efficient application in pharma biodegradation.

For achieving pharma biodegradation

(A) potent degraders of anthracene and naphthalene either with a pure culture of (1) bacterial isolates[12,13] or (2) with a pure culture of fungal isolates [10] or (3) with consortia of

bacterial isolates [14,15] or (4) with consortia of fungal isolates [13,10,] or (5) with consortia of isolates i.e. Bacteria and Fungi [14] were used in biodegradation of Ciprofloxacin (C), Diclofenac (D), Ibuprofen (I) and Ranitidine (R) that were used as a single substrate (pharmaceutical pollutants). [12,10,17]

(B) potent degraders of anthracene and naphthalene in consortium (as given above) were used in biodegradation of C, D, I and R in which four combination of pharmaceuticals were used as viz. C+D, D+I, I+R & R+C.

Biodegradative activity of the above mentioned isolates to degrade pharmaceutical compounds was determined by using potent isolates i.e. anthracene and naphthalene degraders. The pharmaceutical compounds were incorporated in Czapek-Dox Medium (for fungal isolates) and Bushnell Hass Medium (for bacteria isolates). Using a nichrome wire, potent isolates (2-3 loopful) were streaked to the plates. The plates were incubated at room temperature for 10-15 days. The plates were observed for growth of potent isolates. The growth of degraders corresponded to their ability to biodegrade or consume recalcitrant chemicals.

Results And Discussion:

Bacteria and fungi with their degradability of Polycyclic Aromatic Hydrocarbons were enriched in 1 and 10µg/ml, isolated at 1µg/ml concentration of Polycyclic Aromatic Hydrocarbons, in Mineral Salts Medium medium. Further, in anthracene and naphthalene degraders, the number of fungal isolates obtained (i.e. 19) is more than number of bacterial isolates (i.e. 12) and in that the number of naphthalene degraders are higher than number of anthracene degraders.

Recently, fungi have received considerable attention for their biodegradative potential which is attributed to the enzymes they produce that are involved in lignin breakdown and which degrade a wide range of recalcitrant pollutants, such as Polycyclic Aromatic Hydrocarbons, pharmaceuticals and pesticides. In addition, fungi have an advantage over bacteria in that fungal hyphae can penetrate contaminated soil to reach the Polycyclic Aromatic Hydrocarbons that have spread beyond the top layer of the soil.

Identification of isolates:

Anthracene degrading bacteria B1 *Staphylococcus spp.*, B2 *Aeromonas spp.*, B3 *Serratia spp.*, B4 *Micrococcus spp.*, B5 *Klebsiella spp.* B6 *Vibrio spp.* and fungi F1 *Penicillium spp.*,

F2 *Rhizopus spp.*, F3 *Penicillium spp.*, F4 *Rhizopus spp.*, F5 *Penicillium spp.*, F6 *Aspergillus spp.*, F7 *Penicillium spp.*, F8 *Neurospora spp.* were identified. (Table 1)

Table 1: List of Anthracene degraders.

Isolates	Anthracene degraders
B1	<i>Staphylococcus spp.</i>
B2	<i>Aeromonas spp.</i>
B3	<i>Serratia spp.</i>
B4	<i>Micrococcus spp.</i>
B5	<i>Klebsiella spp.</i>
B6	<i>Vibrio spp.</i>
F1	<i>Penicillium spp.</i>
F2	<i>Rhizopus spp.</i>
F3	<i>Penicillium spp.</i>
F4	<i>Rhizopus spp.</i>
F5	<i>Penicillium spp.</i>
F6	<i>Aspergillus spp.</i>
F7	<i>Penicillium spp.</i>
F8	<i>Neurospora spp.</i>

Table 2: List of Naphthalene degraders.

Isolates	Naphthalene degraders
B7	<i>Erwinia spp.</i>
B8	<i>Micrococcus spp.</i>
B9	<i>Staphylococcus spp.</i>
B10	<i>Vibrio spp.</i>
B11	<i>Vibrio spp.</i>
B12	<i>Staphylococcus spp.</i>
F9	<i>Aspergillus spp.</i>
F10	<i>Aspergillus spp.</i>
F11	<i>Penicillium spp.</i>
F12	<i>Neurospora spp.</i>
F13	<i>Penicillium spp.</i>
F14	<i>Aspergillus spp.</i>
F15	<i>Slime mold</i>
F16	<i>Coelomycetes spp.</i>
F17	<i>Rhizopus spp.</i>
F18	<i>Penicillium spp.</i>
F19	<i>Neurospora spp.</i>

Naphthalene degrading bacteria B7 *Erwinia spp.*, B8 *Micrococcus spp.*, B9 *Staphylococcus spp.*, B10 *Vibrio spp.*, B11 *Vibrio spp.*, B12 *Staphylococcus spp.* and fungi F9 *Aspergillus spp.*, F10 *Aspergillus spp.*, F11 *Penicillium spp.*, F12 *Neurospora spp.*, F13 *Penicillium spp.*, F14 *Aspergillus spp.*, F15 *Slim mold*, F16 *Coleomyces spp.*, F17 *Rhizopus spp.*, F18 *Penicillium spp.*, F19 *Neurospora spp.* were identified. (Table 2)

Biodegradation of Pharmaceuticals:

Potent degraders of anthracene and naphthalene either in single or in consortium were used in biodegradation of Ciprofloxacin (C), Diclofenac (D), Ibuprofen (I) and Ranitidine (R) either single or in combination (C+D, D+I, I+R & R+C). In this study, the growth of micro-organisms corresponded to the ability of these microorganisms to consume recalcitrant chemicals.

It was found that Ciprofloxacin was degraded by not only pure fungal culture i.e. F1, F7 & F9 but

also consortia of fungi i.e. F4+F5, F6+F7, F10+F11 & F13+F14 and also consortia of bacteria and fungi B7+F9, B9+F11, B10+F12 & B11+F13. However, Diclofenac was degraded by pure culture of bacteria B3, pure culture of fungi F5, consortia of bacteria B6+B1 and consortia of bacteria & fungi B6+F6 and B9+F11.

While Ibuprofen degradation was carried out by pure culture as well as by consortia of micro-organisms involves pure culture of bacteria B5, pure culture of fungi F14, consortia of bacteria B3+B4 & B7+B8 by consortia of fungi F5+F6, F11+F12 and consortia of bacteria & fungi B5+F5, B8+F10, B10+F12 & B11+F13. Similarly, Ranitidine degradation was carried out by pure culture of bacteria B1, B8 & B12, pure culture of fungi F4, F16 & F19, consortia of bacteria B1+B2 & B9+B10, consortia of fungi F1+F2, F10+F11 & F18+F19, consortia of bacteria & fungi B4+F4, B5+F5, B6+F6, B7+F9, B8+F10, B10+F12 & B12+F14. (Graph 1 & Table 3, 4, 5, 6, 7)

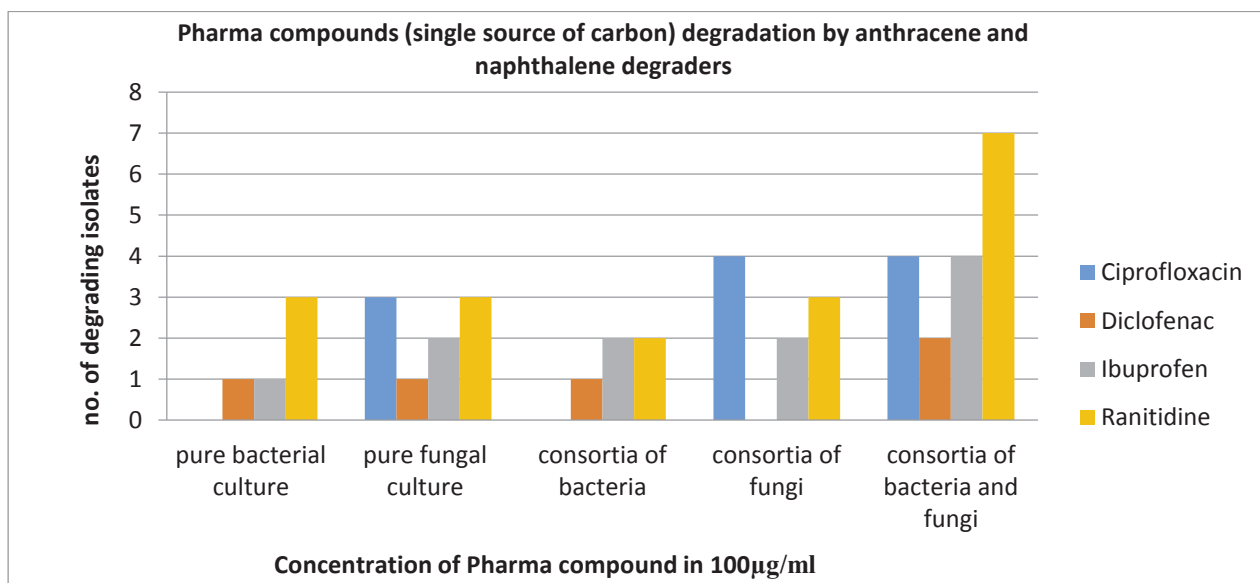


Table 3: Pharma compounds degradation by bacterial isolates.

Used potent Anthracene or naphthalene degraders: Bacteria

Anthracene degraders							Naphthalene degraders					
	1	2	3	4	5	6	7	8	9	10	11	12
C	-	-	-	-	-	-	-	-	-	-	-	-
D	-	-	+	-	-	-	-	-	-	-	-	-
I	-	-	-	-	+	-	-	-	-	-	-	-
R	+	-	-	-	-	-	-	+	-	-	-	+

KEY: + = Growth; - = No growth

Table 4: Pharma compounds degradation by fungal isolates.

Used potent Anthracene or naphthalene degraders: Fungi

Anthracene degraders									Naphthalene degraders										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
C	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-
D	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+

Table 5: Pharma compounds degradation by consortia of bacterial isolates.

Used potent Anthracene or naphthalene degraders: Bacteria

Anthracene degraders							Naphthalene degraders						
	1+2	2+3	3+4	4+5	5+6	6+1	7+8	8+9	9+10	10+11	11+12	12+7	
	-	-	-	-	-	-	-	-	-	-	-	-	
	-	-	-	-	-	+	-	-	-	-	-	-	
	-	-	+	-	-	-	+	-	-	-	-	-	
	+	-	-	-	-	-	-	-	+	-	-	-	

Table 6: Pharma compounds degradation by consortia of fungal isolates

Used potent Anthracene or naphthalene degraders: Fungi																			
Anthracene degraders								Naphthalene degraders											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	3	4	5	6	7	8	1	10	11	12	13	14	15	16	17	18	19	9
C	-	-	-	+	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-
D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-
R	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-

Table 7: Pharma compounds degradation by consortia of bacterial and fungal isolates.

Used potent Anthracene or naphthalene degraders: Bacteria + Fungi													
Anthracene degraders							Naphthalene degraders						
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	
	+	+	+	+	+	+	+	+	+	+	+	+	+
	F1	F2	F3	F4	F5	F6	F9	F10	F11	F12	F13	F14	
C	-	-	-	-	-	-	+	-	+	+	+	-	
D	-	-	-	-	-	+	-	-	+	-	-	-	
I	-	-	-	-	+	-	-	+	-	+	+	-	
R	+	-	-	+	+	+	+	+	-	+	-	+	

During the study of single substrate biodegradation it was seen that pure bacterial cultures were unable to degrade the Ciprofloxacin. This substrate was degraded by some pure fungal cultures, consortia of fungi and consortia of bacteria & fungi. However, Diclofenac was degraded by pure culture of bacteria, consortia of bacteria and consortia of bacteria & fungi.

While Ibuprofen degradation was carried out by pure culture of bacteria and fungi as well as by consortia of bacteria, consortia of fungi and consortia of bacteria & fungi. Similarly, Ranitidine degradation was carried out by pure culture of bacteria and fungi as well as by

consortia of bacteria, consortia of fungi and consortia of bacteria & fungi.

Diclofenac & Ibuprofen were not used as a co-substrate by any of the isolated micro-organisms. While, Ibuprofen & Ranitidine co-substrate were appeared to degrade not only by pure culture of fungi F12 but also by consortia of bacteria B8+B9 and consortia of bacteria & fungi B5+F5.

However, it can be seen that when C & D were used as co-substrate, they were degraded only by pure culture of bacteria B3, pure culture of fungi F1, F6, F9 & F14, consortia of fungi F10+F11 and consortia of bacteria & fungi B4+F4, B8+F10 & B9+F11. Similarly, R&C degradation were

carried out by pure culture of bacteria B₄, B₆ of bacteria & fungi B₁+F₁, B₇+F₉, B₈+F₁₀ & B₁₂, pure culture of fungi F₃, F₁₀ & F₁₆, & B₁₁+F₁₃. (Graph 2 & Table 8, 9, 10, 11, 12) consortia of fungi F₄+F₅, F₁₃+F₁₄ and consortia

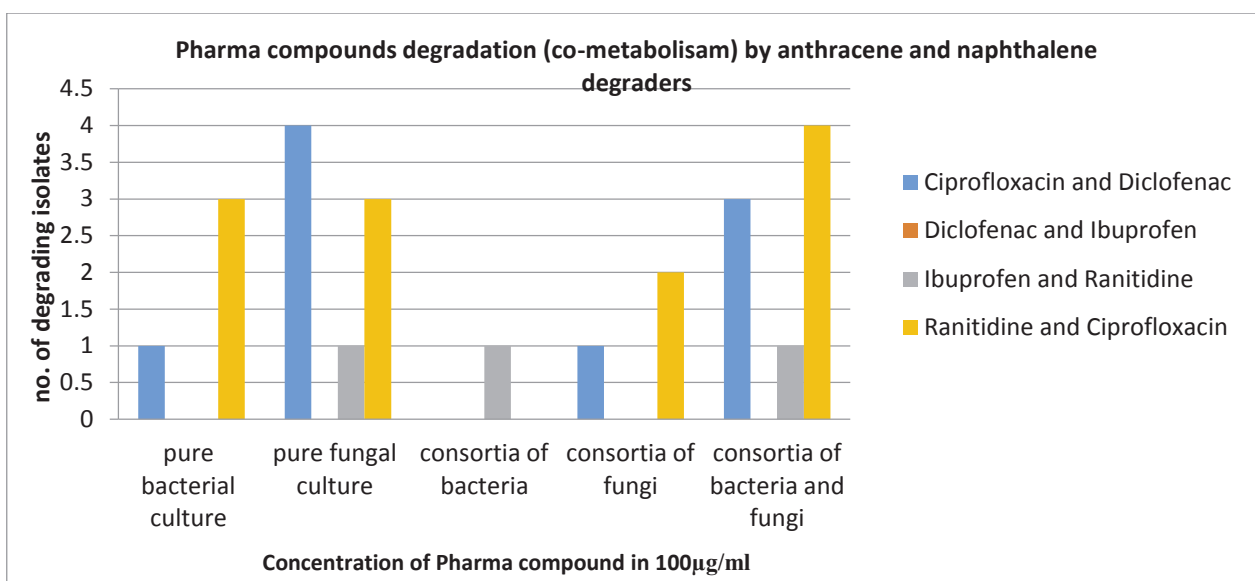


Table 8: Pharma compounds as a co- substrate degradation by bacterial isolates.

Used potent Anthracene or naphthalene degraders: Bacteria												
	Anthracene degraders						Naphthalene degraders					
	1	2	3	4	5	6	7	8	9	10	11	12
C+D	-	-	+	-	-	-	-	-	-	-	-	-
D+I	-	-	-	-	-	-	-	-	-	-	-	-
I+R	-	-	-	-	-	-	-	-	-	-	-	-
R+C	-	-	-	+	-	+	-	-	-	-	-	+

Table 9: Pharma compounds as a co-substrate degradation by fungal isolates.

Used potent Anthracene or naphthalene degraders: Fungi																				
	Anthracene degraders								Naphthalene degraders											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
C+D	+	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	
D+I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
I+R	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
R+C	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	

Table 10: Pharma compounds as a co-substrate degradation by consortia of bacterial isolates.

Used potent Anthracene or naphthalene degraders: Bacteria												
Anthracene degraders						Naphthalene degraders						
	1	2	3	4	5	6	7	8	9	10	11	12
	+	+	+	+	+	+	+	+	+	+	+	+
	2	3	4	5	6	1	8	9	10	11	12	7
C+D	-	-	-	-	-	-	-	-	-	-	-	-
D+I	-	-	-	-	-	-	-	-	-	-	-	-
I+R	-	-	-	-	-	-	-	+	-	-	-	-
R+C	-	-	-	-	-	-	-	-	-	-	-	-

Table 11: Pharma compounds as a co-substrate degradation by consortia of fungal isolates.

Used potent Anthracene or naphthalene degraders: Fungi																			
Anthracene degraders								Naphthalene degraders											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	3	4	5	6	7	8	1	10	11	12	13	14	15	16	17	18	19	9
C+D	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
D+I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I+R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R+C	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-

Table 12: Pharma compounds as a co-substrate degradation by consortia of bacterial and fungal isolates.

Used potent Anthracene or naphthalene degraders: Bacteria + Fungi												
Anthracene degraders						Naphthalene degraders						
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
	+	+	+	+	+	+	+	+	+	+	+	+
	F1	F2	F3	F4	F5	F6	F9	F10	F11	F12	F13	F14
C+D	-	-	-	+	-	-	-	+	+	-	-	-
D+I	-	-	-	-	-	-	-	-	-	-	-	-
I+R	-	-	-	-	+	-	-	-	-	-	-	-
R+C	+	-	-	-	-	-	+	+	-	-	+	-

Different co-substrate i.e. pharma compounds at 100 µg/ml concentration were used as a source of

carbon by many bacteria and fungi. D&I were not used as co-substrate by isolated micro-organisms, meaning, these micro-organisms are unable to degrade the co-substrate D & I. While I& R co-substrate were degraded not only by pure culture of fungi but also by consortia of bacteria and consortia of bacteria & fungi.

However, it can be seen that when C & D used as co-substrate, they were degraded not only by pure culture of bacteria and fungi but also by consortia of fungi and consortia of bacteria & fungi. The same criteria were required for R & C degradation but the micro-organisms involved in R&C degradation are different.

Up to 60% of the administered dose of a pharmaceutical is excreted unmetabolized in urine or stool and discharged into domestic wastewater. About 26 metric tons of pharmaceutical waste is disposed annually down the drain in the US alone. Studies in Austria, Brazil, Canada, Croatia, Germany, Greece, Italy, Spain, Switzerland, The Netherlands, the UK and the US have found more than 80 pharmaceutical compound including antibiotics, painkillers, hormones, tranquilizers, anti-inflammatory, chemotherapeutic, antiepileptic and hypolipidemic drugs in waterways at ng/l to µg/l levels, which is within the biologically active range for many of these drugs.[11]

For biodegradation of such pharma little more bacteria and fungi have been isolated so far, additionally, very little is known about the biodegradation of pharma by consortium of micro-organism. Many polycyclic aromatic hydrocarbons degrading microorganism including bacteria, fungi and actinomycetes exhibits broad biodegradative profile either in single or in consortium. Hence successfully, here anthracene and naphthalene biodegraders were used for checking their biodegradative profile in biodegradation of Ciprofloxacin, Diclofenac, Ibuprofen and Ranitidine, qualitatively.

Conclusions:

To date, relatively little is known about the consortia which is used for biodegradation of pharma compounds. This has partially been accomplished by this project. It appears that few of the anthracene and naphthalene degraders

(i.e. isolated at **1 µg/ml**) either in pure culture or in consortium are able to degrade the recalcitrant pharma compounds i.e. Ciprofloxacin, Diclofenac, Ibuprofen and Ranitidine either as a single substrate or as a co-substrate in **100 µg/ml** concentration, to some extent.

Very few of Polycyclic Aromatic Hydrocarbons (PAHs) degraders either in pure culture or in consortium are able to degrade the mentioned recalcitrant pharma compounds when they are used as co-substrate.

Based on our findings and previous reports, increasing biodegradation of xenobiotic by co-metabolisms had been confirmed. In Australia, research study investigated the biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons (PAHs) in liquid media and soil by bacteria (*Stenotrophomonas maltophilia* VUN 10,010 and bacterial consortium VUN 10,009) and a fungus (*Penicillium janthinellum* VUO 10,201) that were isolated from separate creosote- and manufactured-gas plant-contaminated soils. The bacteria could use pyrene as their sole carbon and energy source in a basal salts medium (BSM) and mineralized significant amounts of benzo[a]pyrene cometabolically when pyrene was also present in BSM.

In Germany's research study, Ciprofloxacin (CIP), a fluoroquinolone's degradation by basidiomycetous fungi was studied by monitoring ¹⁴CO₂ production from [¹⁴C]CIP in liquid cultures.

It can be concluded that pure fungal culture, consortia of fungi and consortia of bacteria & fungi are the best option for the degradation of mentioned pharma compounds. In bacteria *Staphylococcus spp.*, *Micrococcus spp.* & *Vibrio spp.* and in fungi *Penicillium spp.*, *Aspergillus spp.*, *Rhizopus spp.* & *Neurospora spp.* were highly active either in single or in consortium. This study will further lead to quantitative studies for biodegradation of pharmaceutical pollutants by using selected potent isolates, optimization studies of the biodegradation process as well as evaluation studies for the mechanism of degradation (enzyme involved in the process) and toxicity of

metabolites generated.

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