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Bioremediation of DDT contaminated soil samples using dairy waste manure prepared from spoiled dairy products

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Abstract : *DDT has potential to cause pollution in the environment which adversely affect the environmental balance and cause various types of diseases in human beings, plants and animals.DDT and its metabolites are known to be present and accumulating at numerous sites around the world.Considering the potential for negative effects due to its contamination, it is necessary to determine effective methods of remediation. The purpose of this study was to investigate the ability of certain microorganism obtained from spoiled dairy products to degrade 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT). 11 Different bacteria such as Coccobacillus, Staphylococcus, Bacillus, Streptococcus and Coccus and 4 different fungi such as Aspergillus, Mucor,Rhizopus and Penicilliumwere isolated from spoiled dairy products such as curdled milk,whey,spoiled curd and cow dung which shows the potencyof utilizing DDT as sole source of carbon and converting DDT to its various substituents which are less toxic to environment.Compost was prepared by the use of these dairy products.The degradation of DDT in soil samples by pure dairy isolates and in prepared compost were analysed and estimated by using FTIR. It was observed that the bacteria and fungi isolated from the dairy waste and prepared compost has the capability to degrade DDT as DDT was converted to DDD,DDNU,DDOH,DDMS.*

Key words:- *Nutrient Agar, Potato Dextrose Agar, Biochemical tests, Minimal media, Composting, Potting, Microbial degradation, HPLC, FTIR.*

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Introduction :

DDT i.e. 1, 1, 1 – trichloro-2, 2- bis (p-chlorophenyl) ethane is an organochlorine insecticide that was first synthesized in 1874. DDT has been used worldwide in pest control, agriculture and forestry industry since World War II. The reason why DDT was so widely used was because it is effective, relatively inexpensive to manufacture, and lasts a long time in the environment. DDT works by affecting the nervous system by interfering with normal nerve impulses. However, it was canceled in 1972 by the United States Environmental Protection Agency (EPA) because it persists in the environment. The soil half-life for DDT is from 2 to 15 years. It accumulates in fatty tissues, and can cause adverse health effects on wildlife and human including reproductive and birth effects. Considering the potential for negative effects due to this contamination,it is necessary to determine effective methods of remediation.

Biodegradation of DDT largely involves co-metabolism in which microbes growing at the expense of a growth substrate are able to transform DDT without deriving any nutrient or energy for growth from the process. Most reports indicate that DDT is reductively dechlorinated to DDD under reducing conditions Dairy waste including spoiled dairy products like whey, curdled milk, spoiled curd, cow dung, wash water etc. comprises of diverse range of microbes including bacteria *(Pseudomonas species, Klebsiella species, Serretia species, Bacillus species etc), fungi (Penicillium sp., Aspergillus sp., Mucor sp., Rhizopus sp.* etc) and many more, which can acts on this pesticide resulting in their biodegradation. The application of composted organic residues to soils has been addressed in depths because such residues increase soil fertility and organic matter content. (Puglisi et al., 2007).

Therefore, degradation of pesticides can be easy, cost-effective as well as eco-friendly using dairy waste manure including spoiled dairy products which would also further enhances the quality of soil and quantity of the yield.

The main purpose of this study is to isolate the number of different microbe present in common dairy waste such as bacteria and fungi which have ability to degrade DDT present in soil in minimum possible period of time and to observe the effect on the physiochemical properties of soil.

Materials and Methods :

The present research work was conducted in the Department of Industrial Microbiology, Patna women's college, R.M.R.I.M.S,Agamkuan and Central Soil Testing Laboratory,Mitthapurduring the period of July to October 2016.

Two soil samples R1 and R2 were collected (approx. 15 cm deep) from the rice field of Belwar and Chitkohra respectively. Four different dairy samples which include whey, curddled milk,spoiled curd and cow dung where collected from the common source. The soil sample was then tested for the absence of DDT and known concentration of DDT was mixed into the soil sample (Purnomo et al., 2008).

Two soil samples (R1and R2) and four dairy samples cow dung, whey, curddled milk and spoiled curd were serially diluted in normal salineand spreaded on NA and PDA media plates and incubated at 37C for 24 h and 26C for 24-48 h respectively.

Different bacterial strains were isolated from the soil and dairy sample and were characterized on the basis of colony characteristics (colour, texture, elevation, colony no.) and gram staining and are identified on the basis of different bio-chemical tests like Amylase test, Casein hydrolysis test, H_2S production test, Sugar (carbohydrate) utilization test, litmus milk test, urease test, IMVic test, Catalase test (Aneja, 2013).

Different fungal strains were isolated and identified based on the appearance, mycelia, spores and colour according to lactophenol cotton blue test (Aneja, 2013).

Composting was done in vessel (Ed. Rynk, Robert, 1992). Soil (R1 and R2, in equal proportion) and cow dung was mixed evenly in ratio 2:1 in a container and irrigated with whey. It was covered with cellophane sheet having pores which allow aeration and left for maturation. The whole mixture was mixed mechanically at fixed interval of time.

The Isolated pure strains of dairy wastes were streaked on sterilized minimal media containing DDT as a sole source of carbon, using spread plate technique respectively. Plates were incubated at 35°C for 24 – 48 h (KaviKarunya and Reetha, 2012).

The isolates with best growth at concentration was grown in minimal broth containing same concentration of DDT and incubated at 35°C in shaker incubator. Absorbance was taken for bacterial and fungal growth at regular time intervals of 0, 3, 6, 14 and 17 days at 600 nm using U.V visible spectrophotometer (IS: 5864, 1983).

Analysis of physical and chemical properties of soil sample (R1 and R2) and prepared compost was done at Central Soil Testing Laboratory, Mithapur, Patna.

Physical properties include temperature, pH and electrical conductivity using pH meter and conductivity meter (KaviKarunya and Reetha, 2012).

Chemical properties include concentration of P_2O_4 by Olsen method, organic carbon by Walkley and Black method, Potash by ammonium acetate method, available nitrogen, C: N ratio and micronutrients (Zn, Cu, Mn and Fe) using atomic absorption spectrophotometer and flame spectrometer.

In order to degrade the available known amount (0.2/ml) of DDT in soil sample, DDT contaminated soil samples were potted and inoculated with pure dairy isolates and prepared compost.First thepots were wiped with 95 % ethanol, labelled and exposed to U.V radiations in order to surface sterilise. It was then filled with the two soil samples R1 and R2 containing known concentration of DDT and inoculated with pure dairy isolates and compost in following manner as given in Table 1 (KaviKarunya and Reetha, 2012). Rice seeds were sown into each.Each pot was irrigated at certain period of time with equal amount of tap water and growth of plant was observed. After every week, no. of plantlets was counted and length of root and shoot was measured in each pot.

Table 1. Content of different pots

S.N.	Particulars	Content
1.	Pot 1	R1 + 0.2ml/ml DDT + compost
2.	Pot 2	R2 +0.2ml/ml DDT + compost
3.	Pot 3	R1 + 0.2ml/ml DDT + pure isolates
4.	Pot 4	R2 + 0.2ml/ml DDT + pure isolates
5.	Pot 5	Autoclaved R1 +0.2ml/ml DDT + compost
6.	Pot 6	Autoclaved R2 + 0.2ml/ml DDT + compost
7.	Pot 7	Autoclaved R1 + 0.2ml/ml DDT+pure isolates
8.	Pot 8	Autoclaved R2 + 0.2ml/ml DDT+ pure isolates
9.	Pot 9	R1 + 0.2ml/ml DDT
10.	Pot 10	R2 + 0.2ml/ml DDT

In order to estimate the degradation of DDT in soil samples by pure dairy isolates and microbes in prepared compost, small amount of soil mixture from each pot was taken out at 25 days and analysed by FTIR (Ming and Russell, 2001).

Results and Discussion :

Different bacterial isolates obtained from dairy waste and soil were identified and characterized on the basis of morphology, gram's reaction and different biochemical tests.The biochemical tests results for 13 bacterial isolates are shown in Table 2.

Table 2. Biochemical test results of 13 isolates

Isolates	Amylase test	H2S production	Urease test	Sugar utilisation	Indole production	MR test	VP test	Citrate utilisation
1	+	_	_	A	_	_	+	_
2	+	_	_	A	_	+	_	_
3	_	_	_	A	_	+	+	_
4	_	_	+	_	_	_	_	+
5	+	_	_	A	_	+	_	_
6	+	_	_	A	_	+	_	+
7	_	_	_	_	_	_	_	_
8	_	_	_	_	_	_	_	+
9	_	_	_	A	_	+	_	_
10	_	_	_	A	_	+	_	+
11	_	_	_	A	_	+	+	_
12	_	_	_	_	_	+	+	_
13	+	_	_	A	_	+	_	_

After biochemical tests, the 13 isolated cultures were identified on basic of Bergey's manual of determinative bacteriology.The bacterial isolates were identified as different species of *Bacillus, Staphylococcus, Streptococcus, Coccus and Coccobacillus.*

Identification of Fungi

Different types of fungi were isolated from all the samples as shown in Table 3.

Table 3. Results of fungi isolates from soil samples and spoiled dairy products

Sample	Types of colonies	Colony characteristics			Organism	Source
		Color	Margin	Texture		
Soil	3	Green	Irregular	Cottony	*Mucor*	R1
		Green	Irregular	Cottony	*Aspergillusflavus*	R2
		White	Irregular	Cottony	*Aspergillusfumigatus*	R1
Spoiled dairy products	4	Green	Irregular	Cottony	*Mucor*	Curdled milk
		White	Irregular	Cottony	*Rhizopus*	Cow dung
		Dark green	Irregular	Cottony	*Aspergillus*	Curdled milk, Cow dung
		Green	Irregular	Cottony	*Penicillium*	Curdled milk, Cow dung
Compost	3	Green	Irregular	Cottony	*Mucor*	
		White	Irregular	Cottony	*Rhizopus*	
		Green	Irregular	Cottony	*Penicillium*	Compost
		Green	Irregular	Cottony	*Aspergillusflavus*	
		White	Irregular	Cottony	*Aspergillusfumigatus*	

Different types of fungi were isolated from all the samples as shown in Table 3. From soil sample R1, *Mucor* and *Aspergillus fumigatus* were isolated while from soil sample R2*, Aspergillus flavus* was isolated. *Mucor, Rhizopus, Penicillium, Aspergillus flavus* and *Aspergillus fumigatus* were isolated from prepared dairy waste compost. *Mucor, Aspergillus* and *Penicillium* were isolated from curdled milk while *Rhizopus, Aspergillus* and *Penicillium* were isolated from cow dung. No fungi were isolated from whey and spoiled curd.

Table 4. Optimization of isolated bacterial and fungal strains of dairy samples at 0.2 µl/ml DDT in minimal broth

S. No.	Sample	Minimal broth (ml)	DDT (0.2µl/ ml)	Absorbance at 600 nm (3 days incubation)	Absorbance at 600 nm (6 days incubation)	Absorbance at 600 nm (14 days incubation)	Absorbance at 600 nm (19 days incubation)
1.	Whey bacteria	100	0.2	0.049	0.056	0.163	0.012
2.	Cow dung bacteria	100	0.2	0.100	0.105	0.549	1.758
3.	Curdled milk bacteria	100	0.2	0.030	0.071	0.471	0.195
4.	Spoiled curd bacteria	100	0.2	0.188	0.420	1.705	1.684
5.	*Penicillium*	100	0.2	0.067	0.083	0.119	0.044
6.	*Aspergillus*	100	0.2	0.019	0.022	0.421	0.409
7.	*Mucor*	100	0.2	0.121	0.176	0.958	0.769
8.	*Rhizopus*	100	0.2	0.104	0.152	1.082	1.052

Bacterial and fungal isolates with high potential to utilize DDT as sole carbon source were cultivated in minimal broth having DDT (0.2µl/ ml). Growth pattern at different interval of time are shown in Table 4. This was found in correlation with the work of KaviKarunya and Reetha (2012).

All the bacterial and fungal isolates are found to have their log phase during 7-14 days, however log phase continues upto 19 days only in case of bacteria isolated from cow dung. Therefore, DDT degradation may have been occurred between 7-14 days.

Results of physical and chemical properties of soil samples, compost soil mixture (after degradation of DDT) are shown in Table 5, 6, 7 and 8.

Table 5. Physical properties of soil samples and compost

S.N.	Samples	pH	Temperature (°C)	Electrical conductivity (decisimon/cm)
1.	R1	4.064	31.3	0.102
2.	R2	7.264	46	0.333
3.	Compost	6.654	52	0.502

Analysis of physical properties of soil samples and compost shows their particular pH, temperature and electrical conductivity by the above mention Table 5.

Table 6. Chemical properties of soil samples and compost

S. No.	Samples	Organic carbon (%)	P_2O_5 (ppm)	K_2O (Kg/ hectare)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Fe (ppm)
1.	R1	0.62	14	4.09	0.842	0.230	41.60	5.102
2.	R2	1.26	43	17.92	1.206	0.138	38.20	7.564
3.	Compost	2.01	87	518	0.250	1.158	1.644	2.962

Analysis of chemical properties of soil samples and compost shows the amount of micro and macronutrient present in the samples and compost by the above mentioned Table 6.

Table 7. Physical properties of soil mixture (after DDT degradation)

Pot No.	Content (decisimon/cm)	pH	Electrical conductivity
1.	Autoclaved R2 + pure culture + DDT	7.080	0.233
2.	Autoclaved R1 + pure culture + DDT	7.345	0.237
3.	R1 + pure culture + DDT	6.499	0.123
4.	R2 + pure culture + DDT	6.744	0.224
5.	Autoclaved R2 + compost + DDT	7.085	0.276
6.	R2 + compost + DDT	7.058	0.182
7.	Autoclaved R1 + compost + DDT	6.901	0.200
8.	R1 + compost + DDT	6.830	0.140

Analysing the soil mixture after degradation shows the enhanced physical characteristics of pot – 2, 3, 8 which contains soil sample R1 by the above mentioned Table 7.

Table 8. Chemical properties of soil mixture (after DDT degradation)

Pot. No.	Contents	Organic carbon (%)	P_2O_5 (ppm)	K_2O (Kg/ hectare)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Fe (ppm)
1.	Autoclaved R2 + pure culture + DDT	0.765	29	380	0.654	0.460	11.15	25.12
2.	Autoclaved R1 + pure culture + DDT	0.435	33	165	1.422	2.730	27.38	26.08
3.	R1 + pure culture + DDT	0.525	30	373	0.857	0.828	13.63	35.86
4.	R2 + pure culture + DDT	0.727	13	424	0.862	2.240	24.64	37.02
5.	Autoclaved R2 + compost + DDT	1.20	24	307	1.470	2.546	31.16	34.46
6.	R2 + compost + DDT	1.100	22	210	1.480	1.012	20.34	35.90
7.	Autoclaved R1 + compost + DDT	1.310	13	209	1.052	2.546	30.60	35.24
8.	R1 + compost + DDT	0.630	18	238	1.290	0.920	27.06	36.08

Analysing the soil mixture after degradation shows the enhanced chemical characteristics of soil in all the pot by the above mentioned Table 8.

Length of root and shoot of theplantlets in each pot are shown in Table 9. However pot 1, 4, 5 and 7 showed no plant growth.

Table 9. Length of root and shoot of best grown plantlets of different pots.

S.N.	Source of plantlet	Length of root (cm)	Length of shoot (cm)
1.	Pot 2	4	35.5
2.	Pot 3	7	11
3.	Pot 6	1.1	2.2
4.	Pot 8	2.2	9.8
5.	Pot 9	3	6.5

The Figure 1 shows the standard graph obtained from 76% DDT solution (w/v) by FTIR. It shows the presence of functional group. C-Cl (765.55 cm-1), C=C (1633.07 cm-1) & C-H (2962.18 cm-1) present in DDT.

Figures 2, 3, 4, 5 show the graphs obtained from the isolates of cow dung and curdled milk in minimal broth containing 0.2µl/ ml of DDT incubated for 19 days, showing the maximum potential to degrade DDT to DDNU, which is one of the intermediate in microbial DDT degradation pathway. Graphs show the presence of functional group C=C and C-H bond (Boul, 1995).

Figures 6, 7, 8, 9, 10, 11, 12 and 13 show the graphs which is obtained after 25 days of incubation of different Pot mixture containing Autoclaved R2 + pure culture + DDT showing that DDT is degraded to DDD and DDOH, DDNU and DDMS which are the intermediates in microbial DDT degradation pathway. The graphs show the presence of functional group C=C, C-Cl, -OH and C-H bonds.

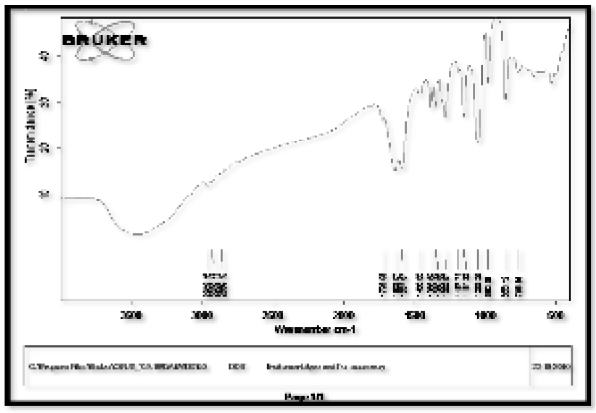

Fig. 1. This graph is obtained from DDT solution showing the presence of functional group present in DDT i.e. C-Cl (765.55 cm-1), C=C (1633.07 cm-1) & C-H (2962.18 cm-1)

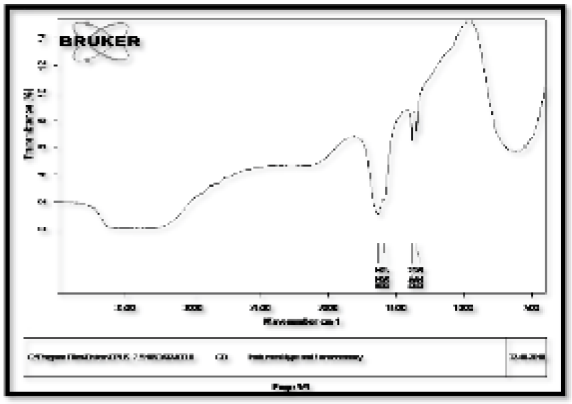

Fig. 2. This graph is obtained from the isolates of cow dung in minimal broth containing 0.2µl/ ml of DDT incubated for 19 days, showing the maximum potential to degrade DDT to DDNU. Graph shows the presence of functional group C=C and C-H bond.

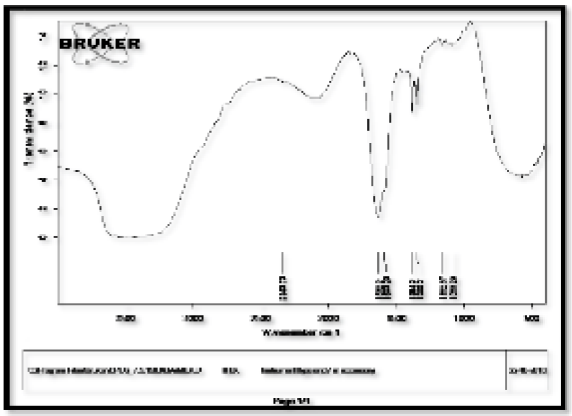

Fig. 3. This graph is obtained from the isolate of curdled milk in minimal broth containing 0.2µl/ ml of DDT incubated for 19 days showing the high potential to degrade DDT to DDNU. Graph shows the presence of functional group C=C and C-H bond.

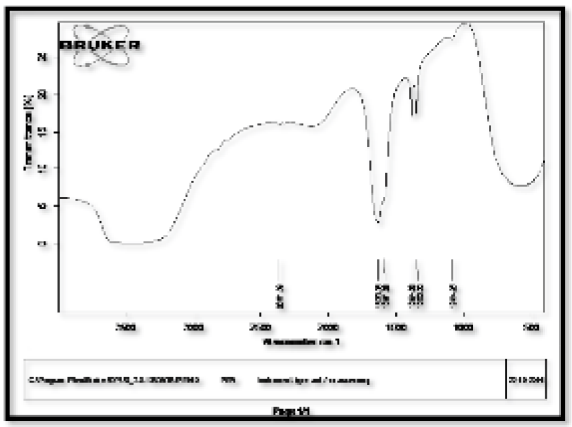

Fig. 4. This graph is obtained from the isolate of *Penicillium* in minimal broth containing 0.2µl/ ml of DDT incubated for 19 days showing the high potential to degrade DDT to DDNU. Graph shows the presence of functional group C=C and C-H bond.

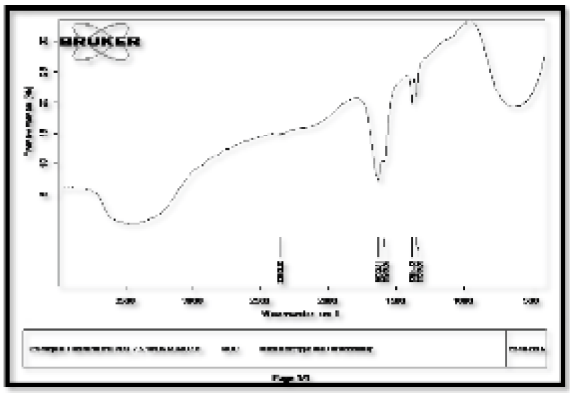

Fig. 5. This graph is obtained from the isolate of *Mucor* in minimal broth containing 0.2µl/ ml of DDT incubated for 19 days showing the high potential to degrade DDT to DDNU. Graph shows the presence of functional group C=C and C-H bond.

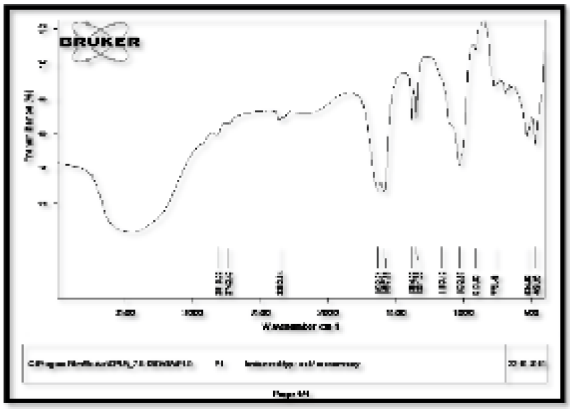

Fig. 6. This graph is obtained after 25 days of incubation of Pot 1 mixture containing Autoclaved R2 + pure culture + DDT showing that DDT is degraded to DDD and DDOH. Graph shows the presence of functional group C=C, C-Cl, -OH and C-H bond.

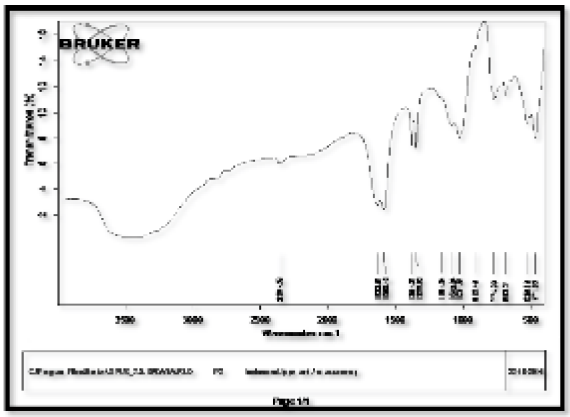

Fig. 7. This graph is obtained after 25 days of incubation of Pot 2 mixture containing Autoclaved R1 + pure culture + DDT showing that the DDT is degraded to DDNU. Graph shows the presence of functional group C=C and C-H bond.

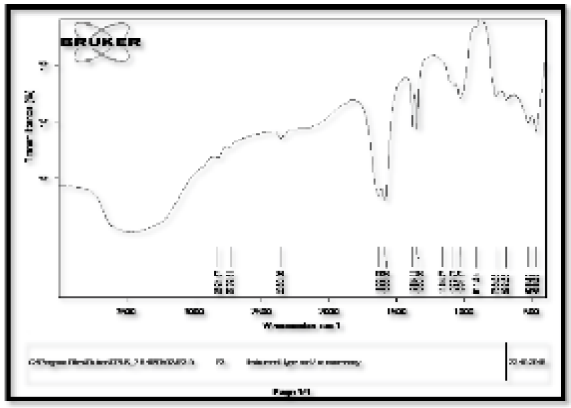

Fig. 8. This graph is obtained after 25 days of incubation of Pot 3 mixture containing R1 + pure culture + DDT showing that the DDT is degraded to DDNU. Graph shows the presence of functional group C=C and C-H bond.

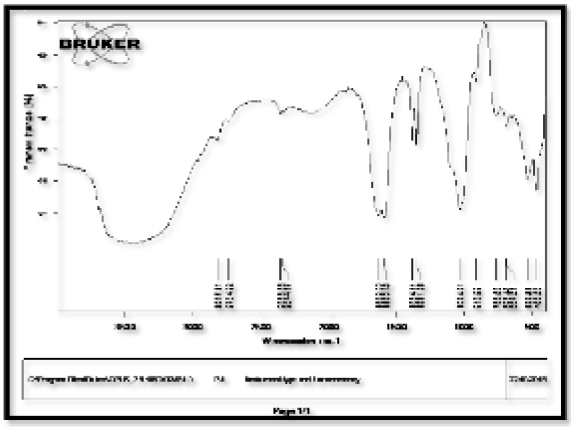

Fig. 9. This graph is obtained after 25 days of incubation of Pot 4 mixture containing R2 + pure culture + DDT showing that the DDT is degraded to DDNU and DDMS. Graph shows the presence of functional group C=C and C-H bond.

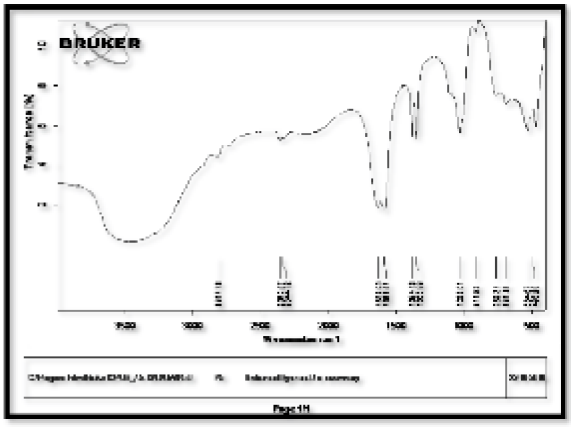

Fig. 10. This graph is obtained after 25 days of incubation of Pot 5 mixture containing Autoclaved R2 + compost + DDT showing that the DDT is degraded to DDNU and DDMS. Graph shows the presence of functional group C=C and C-H bond.

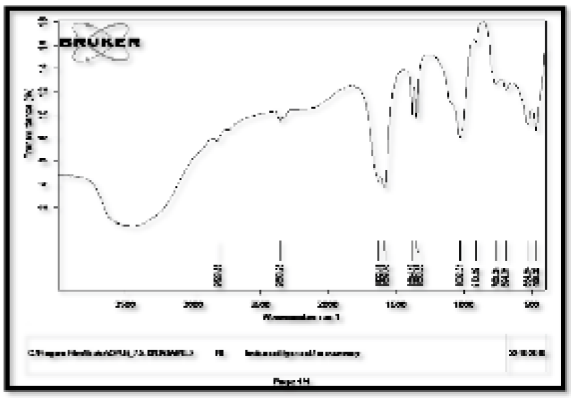

Fig. 11. This graph is obtained after 25 days of incubation of Pot 6 mixture containing R2 + compost + DDT showing that the DDT is degraded to DDNU and DDMS. Graph shows the presence of functional group C=C and C-H bond.

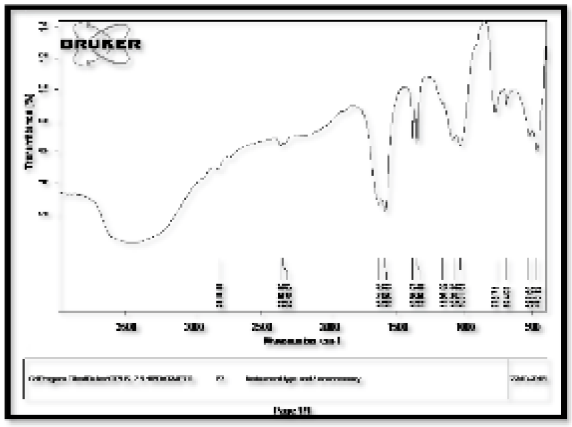

Fig. 12. This graph is obtained after 25 days of incubation of Pot 7 mixture containing Autoclaved R1 + compost + DDT showing that the DDT is degraded to DDNU and DDMS. Graph shows the presence of functional group C=C and C-H bond.

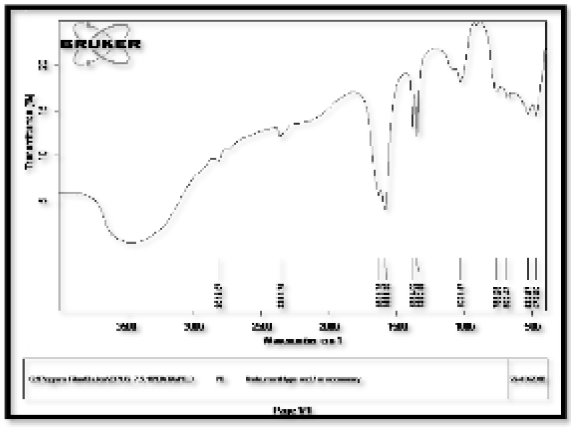

Fig. 13. This graph is obtained after 25 days of incubation of Pot 8 mixture containing R1 + compost + DDT showing that the DDT is degraded to DDNU and DDMS. Graph shows the presence of functional group C=C and C-H bond.

Conclusion :

This study showed the potential of spoiled dairy products and compost prepared from them to degrade soil DDT in DDD, DDMS and DDNU efficiently. Among different dairy waste used, cow dung and curdled milk are found to have higher potential of DDT degradation followed by curd and whey. Bioremediation using dairy waste manure is ecofriendly, cost effective and beneficial for soil as well as crop as this process enhances the fertility of soil and hence will increase the yield.

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