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Efficient Degradation of Micropollutant Triclosan by Microorganisms

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Abstract—*Triclosan [5-chloro-2-(2,4-dichloro-phenoxy)-phenol] is a synthetic antibacterial compound being used in a wide variety of soaps and detergents, as well as in many deodorants, toothpastes, cosmetics, fabrics and plastics. Triclosan is a known endocrine disruptor and a suspected carcinogen. There is good reason to believe that the over use of products such as triclosan has contributed to bacterial resistance in the same way we are cautioned against the use of antibiotics. Triclosan is persistent in nature connecting to the fact that it is being washed down in our drains daily and thus adversely affects the ecosystem. The present study revolves around the isolation and screening of micro-organisms capable of effectively degrading triclosan. The study indicated a potential isolate capable of utilizing triclosan up to 77.97±1.09% added in the minimal media. Upon the use of additional C-sources it was found that Maltose enhances the utilization percentage upto 86.96±0.54%. This might help in the process development of biological treatment for the removal of triclosan effectively from waste water systems.*

1. INTRODUCTION:

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)-phenol) is a common synthetic antimicrobial agent that has been incorporated into more than 700 different industrial and personal care products. These products, including deodorants, soaps, toothpastes, and various plastic products, contain 0.1-0.3% triclosan[6]. It has been mentioned as an endocrine disrupter(ED). It has weak androgenic activity against aquatic organisms and revealed both androgenic as well as estrogenic responses in human breast cancer cells. Triclosan is persistent in nature connecting to the fact that it is being washed down in our drains daily. This may promote the development of antimicrobial resistant micro-organisms which may cause adverse effects on ecosystem.[8]

Waste water is the major source of triclosan to the environment. Incomplete removal of triclosan will occur by conventional waste water treatment process[6]. TCS might transform into chlorodioxins and dibenzofurans, when exposed to UV radiations or heat, and these transformed by products are highly toxic than the parent compound. Various chemical treatment methods are also employed to check the degradation of triclosan. This is also leading to incomplete degradation of triclosan. However the chemical treatment of triclosan could result in the production of toxic products like chlorophenoxy-phenols, chlorophenols, trihalomethanes, and dioxins, which are known to be carcinogenic.[13] By all these studies it is suggested that biodegradation can be effectively used to remove triclosanin waste water treatment process.[6]

The present study includes the isolation, screening and optimization of nutritional parameters to get efficient degradation of triclosan. This might help in the process development of biological treatment for the removal of triclosan effectively from waste water systems.

2. MATERIALS AND METHODS

2.1. Chemicals

Triclosan(98% pure), di-Sodium hydrogen phosphate, Potassium dihydrogen orthophosphate, Sodium chloride, Ammonium chloride, Tryptone soy agar, Nutrient broth, Gram stain kit, Tetramethyl-p-phenylenediamine, trypticase, D-Glucose, Casein, peptone, Glycerol, diPotassium orthophosphate, Magnesium sulphate, Agar, D-Glucose, D-Fructose, D-Lactose, Sucrose, Starch solube purchased from Hi-Media and Irgasan purchased from Sigma-Aldrich. Stock solution of triclosan was prepared in 95% ethanol.

2.2. Isolation of triclosan degrading bacteria

Triclosan degrading bacteria are isolated from waste water ponds in Ranchi i.e., Ranchi lake and drainage near line tank road, Ranchi and soil sample from the garbage, Dispensary, BIT, Mesra. Then the samples are serial diluted up to 10^{-2} dilution. 100µl of each sample was inoculated on Tryptone soy agar (TSA) – triclosan (2%) agar plates by spread plate method. The plates were then kept for incubation at room temperature. The plates were observed for occurrence of colonies with the formation of zone of inhibition around the colonies.

2.3. Screening and selection of triclosan degrading bacteria

The isolates which obtained are then purified on Nutrient Agar plates and then on nutrient agar slants. Such obtained pure cultures are grown in Nutrient broth for 24h at 37^0C. The

screening tests were conducted in 250ml flasks containing 150ml M9 minimal medium (Molecular cloning, A lab manual) – triclosan (2%). The obtained pure broth cultures were inoculated (1% inoculum) in prepared medium for screening. Aliquots(3ml) of culture media were withdrawn for every 24h, centrifuged at 10,000rpm for 20min to separate bacterial cell mass. The clear supernatant was used to measure degradation by using UV-Visible spectrophotometer spectrum at a wavelength range of 400-200nm. The readings around 280nm are taken to calculate degradation percentage. Reading of uninoculated sample is used to calculate Initial concentration where concentration is calculated from the regression equation of standard plot. Degradation percentage can be calculated from the formula given below

$$Degraradation(\%) = \frac{I - F}{I} * 100$$

Where, I = Initial concentration

F = Final concentration

2.4. Characterization of screened and selected isolate

The characterization of screened isolate was based on Bergey's Manual of Determinative Bacteriology.

2.5. Optimization of additional Carbon sources

Degradation experiments were carried out with M9 minimal medium – triclosan (2%) medium containing 1% carbon source (D-Glucose, D-Fructose, D-Lactose, D- Maltose, Sucrose, Glycerol and Starch soluble). The experiment was carried out at static conditions at a constant temperature. Growth was monitored by cell mass. Aliquots(3ml) of culture media were withdrawn for every 24h, centrifuged at 10,000rpm for 20min to separate bacterial cell mass. The clear supernatant was used to measure degradation by using UV-Visible spectrophotometer spectrum at a wavelength range of 400-200nm. The readings around 280nm are taken to calculate degradation percentage. All the experiments were repeated in duplicates and the mean value was taken for analysis.

3. RESULTS AND DISCUSSION

3.1. Isolation of triclosan degrading bacteria

Bacterial strains having triclosan degrading capacity were isolated from waste water ponds in Ranchi i.e., Ranchi lake and drainage near line tank road, Ranchi and soil sample from the garbage, Dispensary, BIT, Mesra. Bacterial colonies were formed on TSA-triclosan (2%) plates showing zones of inhibition around the colonies. Such strains are then taken and then they were purified using NA plates and then on NA slants. Such obtained slants are kept at 4^0C for further use.

Table 1: Table showing that the number of isolates obtained

Sample	No. of isolates obtained
Soil sample from garbage, Dispensary, BIT, Mesra	2
Ranchi lake	1
Drainage near Line tank, Ranchi	1

3.2. Screening and selection of triclosan degrading bacteria

A standard plot was drawn using Irgasan (1mg/ml).

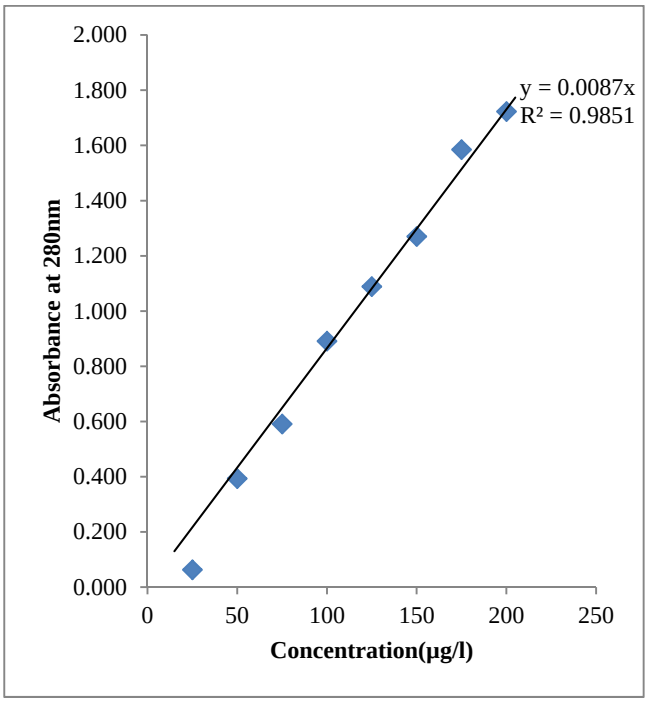

Fig. 1: Standard plot of triclosan

From the above plot the regression equation is obtained as

$$y = 0.008x$$

By using the above equation degradation percentage can be calculated.

Degradation experiments were carried out by inoculating the medium with various isolates, incubating them for 7 days at room temperature and then calculating the percentage degradation at regular intervals i.e., for every 24h.

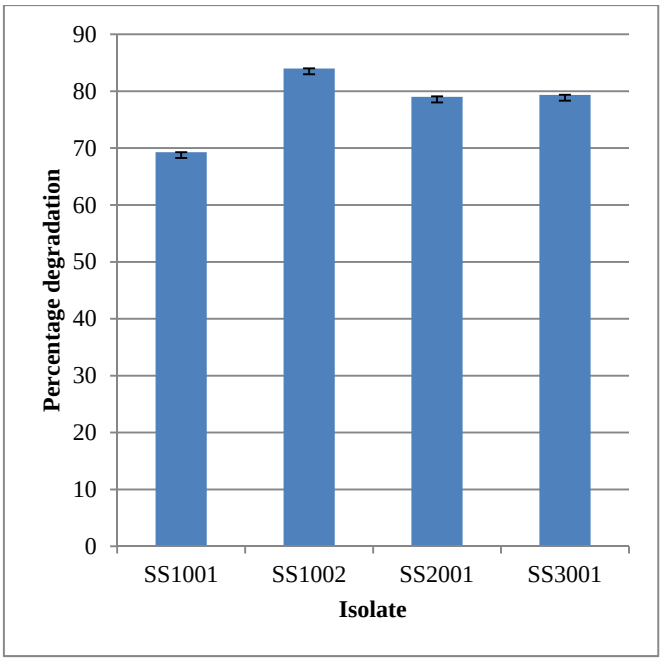

Fig. 2: Diagram showing percentage degradation of triclosan by various bacterial isolates after 7 days of incubation

From the degradation results the isolate selected was SS1002.

3.3. Characterization

3.3.1 Morphological characters

Table 2: Table showing the Morphological characterization of screened isolate SS1002

Growth	
Nutrient Broth	Uniform fine turbidity
Nutrient Agar Plates	Circular with undulate margins
Nutrient agar Slants	Echinulate

3.3.2. Biochemical methods

Table 3: Table showing the Biochemical characterization of screened isolate SS1002

Test	Result
Gram staining	Negative
Shape	Rod shaped
Oxidase	Positive
Glucose fermentation	Negative
Fluorescent diffusible yellow pigment	Positive

So, according to Bergey's Manual of Determinative Bacteriology, the screened isolate might belong to genus *Pseudomonas.*

3.4. Effect of additional Carbon source

The addition of Carbon sources may improve cell growth resulting in more degradation of triclosan. Degradation experiments were carried out by inoculating the medium containing various additional carbon sources with screened isolate, incubating them for 7 days at constant temperature and then calculating the percentage degradation at regular intervals i.e., for every 24h.

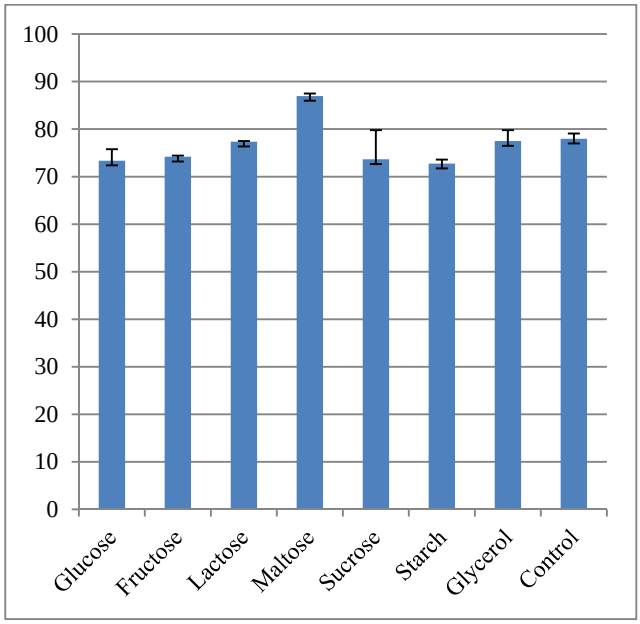

Fig. 3: Diagram showing percentage dagradation of triclosan by using varoius additional C source after 7 days of incubation using isolate SS1002.

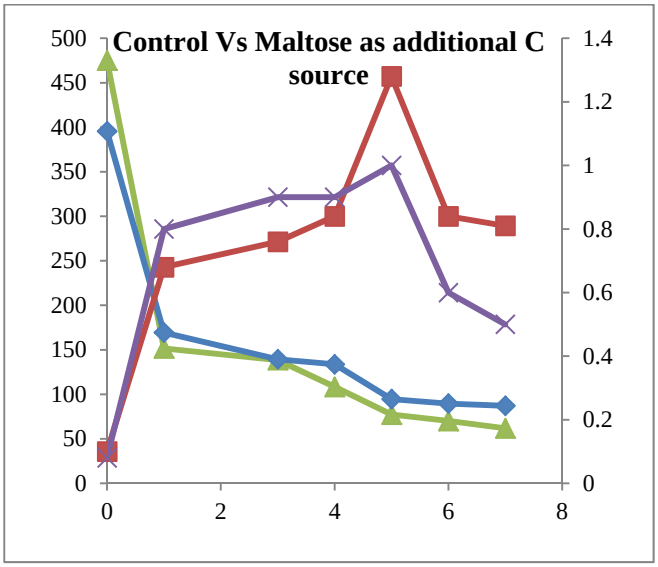

Fig. 4: Diagram showing the increase in cell mass thereby decrease in concentration of triclosan for the media containing maltose as additional carbon source when compared to media without any presence of carbon source.

So, with increase in cell density of the concentration of triclosan is getting decreased showing the utilization of triclosan. This shows that by using Maltose as additional C source, there is increase in cell mass thereby increase in percentage degradation from 77.97 ±1.09 to 86.96 ±0.54 after 7 days of incubation using isolate SS1002.

4. CONCLUSION

In this study, an isolate from soil sample i.e., SS1002, which might belong to genus *Pseudomonas*, can effectively degrade triclosan at a constant temperature. This study also indicates that there is increase in cell mass when maltose is used as additional carbon source. This shows that there is increase in percentage degradation from 77.97 ±1.09 to 86.96 ±0.54 after 7 days of incubation using isolate SS1002. This might help in the process development of biological treatment for the removal of triclosan effectively from waste water systems.

5. ACKNOWLEDGEMENT

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