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BIOREMEDIATION OF INDUSTRIAL EFFLUENTS OF ASANSOL DURGAPUR INDUSTRIAL ZONE AND ITS EFFECT ON DNA

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ABSTRACT
The detailed and core study is been performed on the waste water discharged by various industries in Asansol Durgapur Industrial zone into River Damodar. In this study we have tried to inspect what are the Microbiological and chemical waste which is been discarded in the river by the Steel and Iron Industries, Mining and Paper and pulp mills .Over all our main objective is to study the and characterize the microbes present in the sample of wastewater, secondaly to cram the role of isolated microorganisms from waste water (Durgapur Industrial Area) in waste degradation and also to study its role of the microorganisms from Durgapur industrial wastewater in COD reduction and finally check the damage caused to the synthetic DNA by the industrial waste water.Finally a large number of industrial waste has been noticed example (a) Steel and Iron Industries- as Benzene, Napthalene, Anthracene, Cyanide, Ammonia, Phenol, and Cresol,(b) Mining - Acid-mine drainage (AMD) result from the exposure of sulfide minerals, particularly pyritic and pyrrhotitic minerals and (c) Paper and pulp mills- Sulphuric acids ,dark tans ,slime , piths, and phenolic compounds and moreover bleaching agents effluents which are toxic to aquatic flora and fauna and exhibit a strong mutagenic effect. Finally the Durgapur Industrial Waste Water shows a Color: Light brown, . pH: (7.5+0.2) to (7.5-0.2)., conductivity: 7.77 S/m. It is presumed to have a large amount of solids as indicated by its conductivity value. It contains large amount of phenol and cyanides. Colony characteristics and gram staining shows Negative (Bacillus) and Positive (Coccus). However there is no fungal growth pattern obserevd. It also was observed that in the INDOLE test, all the isolated microorganisms showed negative result indicating that tryptophan was utilized as a nutrient source. COD experiment was done by both the methods i.e. Open reflux method and COD kit technique. But the results were not satisfactory and lastly the Effect of waste water on synthetic DNA was observed as change in the optical density of the Deoxyribonucleic acid (DNA) is been noticed ,which perhaps can be due to the different aromatic ,toxic ,heavy metals , phenolic compounds present in the water. However one more reason can also be Hypothesized, which can be due to some of the very specific compounds which shows a robust property of a Epigenetic, which means addition of methyl group directly to the nucleotides which increases the OD 260 nm of the DNA.Control DNA (100 microgram/ml) OD @260 nm = 0.256 & Treated DNA OD @260nm = 0.957

KEYWORDS: Asansol Durgapur Industrial zone, Acid-mine drainage, conductivity: 7.77 S/m, no fungal growth pattern, 3.5 times increased OD 260 nm of the DNA

INTRODUCTION

The civil world has become incommensurably reliant on industries and developed technologies, ever since industrial revolution took place. Industrial revolution has induced to the indelible increment in the service and consumer market sector and due to that there has been a steady magnification of the adverse effects of industrialization. Industrial effluents are released into the environment from the industrial sites leading to the pollution of water bodies. This act of industries causes incurable consequences for the living world from many points of view, especially regarding public health. These effluents are not only polluting water but also soil and air too. So as a whole this pollutants entering into the environment is a serious problem to be dealt by human mankind (Balcht Aldona *et al*., 1994). The entering of pollutants into our atmosphere has direct and indirect hazardous affects on the living world, irrespective of its physical form. With this context a bottom line can be drawn stating that "It is the entire environment that is vulnerable to pollution. The most significantly dangerous aspect of pollution is its effects on organisms of every kind. Every day there is a huge in equable release of effluents from the industries to the atmosphere leading to accumulation of toxic chemicals or biomagnifications of it. We humans are certainly not excluded from its adverse effect, so slowly and slowly accumulation of toxic levels of chemicals is occurring, which in turn leads to the way towards diseases and even death (Amann *et al*., 1995). Experiments involving cytological effects of industrial effluents, both raw as well as diluted with water either in natural field conditions as in Tamla Nalah water or under controlled dilution in laboratory experiments, showed that various kinds of industrially polluted water produced a

very large number of clearly discernible abnormalities in cell structure and division, ranging from clumping of chromosomes, stickiness, spindle distortion, absence of cell plate formation, dissolution of cell wall, multinucleated cell formation, bridge formation, C–mitotic effect, formation of micronuclei, polyploidy nuclei formation, amitotic cell division, formation of sac like nuclear structures up to complete degeneration of nuclei and cells (S. C. Barman *et al* ., April 2000). Benzene and its phenolics metabolites produce oxidative DNA damage in the H160 cells of bone marrow *in vivo* (Prema Kolachana *et al*., 1993 and Racke *et al*., 1990) Water is the most abundant substance in living systems, making up to 70% or more of the weight of most organisms. Doubtlessly, the first living organism arose in an aqueous environment and the course of evolution has been shaped by the democratic properties of the aqueous medium in which life began.So, it becomes needless to say that water is the most valuable natural resource which needs to be urgently conserved and properly managed.But in the present scenario of industrialization and urbanization, our this very essential and valuable natural resource is getting degraded by the effluent released from various source such as industry, domestic residence, commercial properties and agricultural activities. These effluents encompass a wide range of potential contaminants and concentrations. (Ashok Kumar *et al*., 2010)

Basically there are two major types of waste water source

Point source

Factories, power plants, sewage treatment plants, underground mines, and oil wells are included in these types' sources. They are referred as point source because they discharge pollution from a specific location, such as drain pipes.

Non- point source

This type of sources are scattered or diffused having no precise location where they discharge into particular water body. Non-point source includes run-off from fields, construction sites, roads, streets etc. Industries have played a pivotal role in contaminating the various water bodies (Akpor O. B *et al*., Nov 2010). Which becomes a very serious subject and extremely harmful to the living systems and the environment. Various industries process requires water for various purposes and then they discharge this water directly into the water bodies causing water pollution.

Pollutant discharged from various industries in Asansol-Durgapur Industrial zone:

Steel and Iron Industries

Various types of toxic substances are discharged as industrial effluents contaminate the water bodies. It is known that the iron and steel industries may include gasification products such as Benzene, Napthalene, Anthracene, Cyanide, Ammonia, Phenol, and Cresol together with wide range of more complex organic compounds known collectively as Polyaromatic hydrocarbon, hydraulic oils and particulate solids. To shield public health and environment it is necessary to have knowledge of constituents of concerns in waste water is dispersed into the environment, the transformation and the long term fate of these constituents in treatment process, treatment method that can be used to remove or modify the constituents found in waste water., method for beneficial use for disposal of solids generated by treatment system (Pace N. R. *et al*., 1997). Many industries use physical and chemical methods for the treatment of waste effluent. Nowadays advanced chemical oxidation processes are used to reduce COD and BOD levels, and to remove both organic and oxidize able inorganic compounds, but these are not that much efficient because of cost as well as environmental effects. So microbe based biological methods continue to be the prime choice for efficient and sustainable waste water processing.

Mining

Acid-mine drainage (AMD) results from the exposure of sulfide minerals, particularly pyritic and pyrrhotitic minerals, to atmospheric oxygen and water. AMD directly impacts tens of thousands of kilometers of streams, lakes and estuaries throughout the world. The impacted water bodies tend to have elevated concentrations of metals in the water column or sediments and are also stressed by significant inputs of hydrogen ions. There are several conventional treatment technologies available. The most common is chemical precipitation using lime or other basic substances. These systems produce large volumes of wet sludge that often requires drying facilities to concentrate the metal hydroxide sludge. Wetland treatment systems have also been used for several decades to treat AMD. This treatment system offers less expensive alternative to the conventional chemical precipitation technologies. There still are problems of systems hydraulics and useful life to be addressed.

Paper and pulp mills

It is one of the major industries in our country. The heavy demand for the paper helps in steady expansion of paper industries. Pulp and paper mills are utilizing huge amounts of lignocellulosic components of plants and using chemicals during manufacturing and generally regarded as polluting industries because of huge amount of waste material entered into the environment (Choudhary *et al*., Januarary 2013) Effluents released by pulping and bleaching are amongst the most polluter and are characterized by parameters unique to these waste such as color and organic halides (AOX) (Taseli *et al*., Sept 2004; Fitzsimans *et al*., Sept 1995). The untreated effluents from pulp and paper mills discharged into water bodies, damages the water quality. The brown color imparted to water due to addition of effluents is detectable over long distances. The effluents have high biological and chemical oxygen demands, lignin compounds and their derivatives. The dark brown color is due to the formation of lignin degradation products during the processing of lignocellulosic from paper and pulp manufacture. The undiluted effluents are toxic to aquatic flora and fauna and exhibit a strong mutagenic effect. Furthermore some compounds in the effluents are the aquatic food chain (Sundman *et al*., March 1998). Several methods have been attempted for the removal of color from the pulp and paper mill effluents. These can be classified into physical, chemical and biological methods. Physical and chemical processes are quite expensive and remove high molecular

weight chlorinated lignins, color, toxicants, suspended solids and chemical oxygen demand. But BOD and low molecular weight compound are not removed efficiently (Singh and Singh, 2004). The biological color removal process is particularly attractive since in addition to color and COD it also reduces BOD and low molecular weight chlorolignins (Nagarathnamma et al, 1999, Barton et al, 2004).

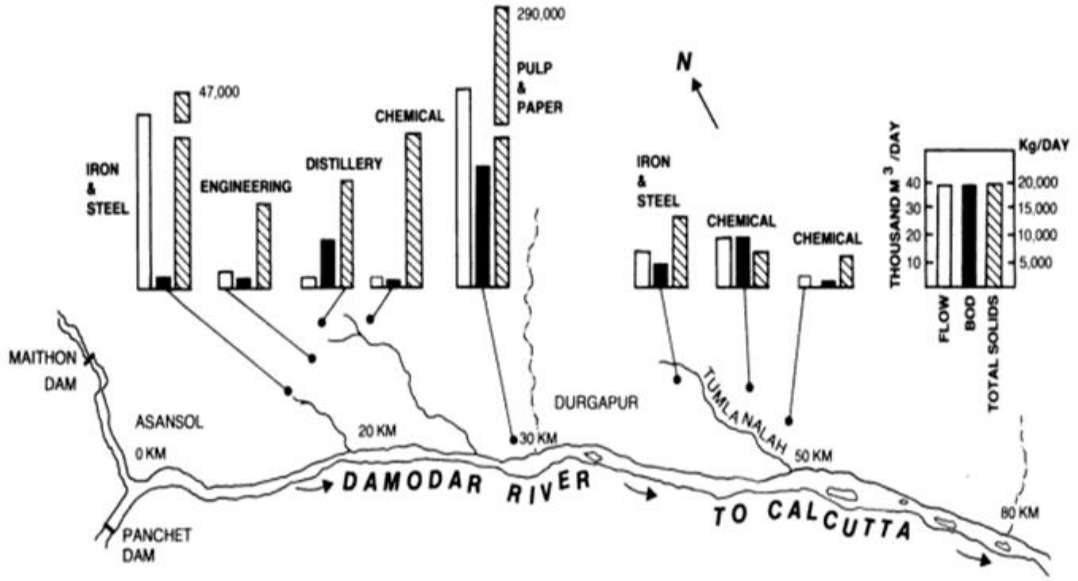

FIGURE 1. The waste water discharged by various industries in Asansol Durgapur Industrial zone into River Damodar

Common types of wastewater treatment methods:-

1) Physical Unit Operations:

Treatment methods in which the application of physical forces predominates are known as physical unit operations. Because most of these methods evolved directly from man's first observations of nature, they were the first to be used for wastewater treatment. Screening, mixing, flocculation, sedimentation, flotation, filtration, and gas transfer are typical unit operations.

2) Chemical Unit Processes:

Treatment methods in which the removal or conversion of contaminants is brought about by the addition of chemicals or by other chemical reactions are known as chemical unit processes. Precipitation, adsorption, and disinfection are the most common examples used in wastewater treatment. In chemical precipitation, treatment is accomplished by producing a chemical precipitate that will settle. In most cases, the settled precipitate will contain both the constituents that may have reacted with the added chemicals and the constituents that were swept out of the wastewater as the precipitate settled. Adsorption involves the removal of specific compounds from the wastewater on solid surfaces using the forces of attraction between bodies (Kielo Haahtela *et al.,* Jan -1981).

3) Biological Unit Processes

Treatment methods in which the removal of contaminants is brought about by biological activity are known as biological unit processes. Biological treatment is used primarily to remove the biodegradable organic substances (colloidal or dissolved) from wastewater. Basically, these substances are converted into gases that can escape to the atmosphere and into biological cell tissue that can be removed by settling. Biological treatment is also used to remove nutrients (nitrogen & phosphorus) from wastewater. With proper environmental control, wastewater can be treated biologically in most cases (Kumar Ashok, et al, 2010).

Overview of industrial wastewater treatment:

Wastewater generated from industrial operations can contain a variety of pollutants, including heavy metals, cyanide, and semi-volatile/volatile organics. Concentrations of these pollutants can impact the performance of a municipal treatment plant by causing pass through or interference which can result in permit violations. As a result, industrial wastewater typically requires separate treatment. It is either pre-treated prior to discharge to an on-site or off-site municipal wastewater treatment facility or it is treated and discharged on site to a permitted discharge point (Racke *et al*., 1990). Example of activities at Air Force Installations generating industrial wastewater requiring treatment include, electroplating, metal finishing, painting, and aircraft maintenance and cleaning. A wide-range of industrial wastewater treatment processes can be employed dependent upon the type and concentration of pollutants and desired effluent quality. These include physical and/or chemical processes which are mentioned below for reference. (Brajesh K. Singh *et al*., 2004).

Primary treatment involves

1. screening- to remove large objects, such as stones or sticks that could plug lines or block tank inlets.
2. Grit chamber-slows down the flow to allow grit to fall out.
3. Sedimentation tank (settling tank or clarifier)- settle able solids settle out and are pumped away, while oils float to the top and are skimmed off.

Secondary treatment involve

sActivated Sludge- The most common option uses microorganisms in the treatment process to break down organic material with aeration and agitation, and then

allows solids to settle out. Bacteria-containing "activated sludge" is continually recirculated back to the aeration basin to increase the rate of organic decomposition. (Pfenning *et al*.,1966)

1. Trickling Filters- These are beds of coarse media (often stones or plastic) 3-10 ft. deep. Wastewater is sprayed into the air (aeration), and then allowed to trickle through the media. Microorganisms attached to and growing on the media, break down organic material in the wastewater. Trickling filters drain at the bottom; the wastewater is collected and then undergoes sedimentation.
2. Lagoons- These are slow, cheap, and relatively inefficient, but can be used for various types of wastewater. They rely on the interaction of sunlight, algae, microorganisms, and oxygen (sometimes aerated). Fig-2

Fig-2, Secondary clarifiers in a wastewater treatment plant:-

After primary and secondary treatment, municipal wastewater is usually disinfected using chlorine (or other disinfecting compounds, or occasionally ozone or ultraviolet light). An increasing number of wastewater facilities also employ tertiary treatment, often using advanced treatment methods (Hazen TC et al 1994). Tertiary treatment may include processes to remove nutrients such as nitrogen and phosphorus, and carbon adsorption to remove chemicals. These processes can be physical, biological, or chemical.

- Cyanide Destruction
- Chromium Reduction
- Metals Precipitation
- Ultrafiltration
- Reverse Osmosis
- Ion Exchange
- Electrodialysis
- Air Stripping

Objective

In view of earlier findings the present study attempts to cover areas of work not covered in the earlier works. It is crystal clear that different studies are going on around the globe and clock for the remedy of various causes and levels of water pollution. People are using physical and chemical methods to degrade the waste present in industrial effluents. Nowadays bioremediations is coming up as one of the most important approach for reducing water pollution level. In this project we have tried to observe the role of isolated bacteria in waste water treatment. The objective is therefore:-

I. To analyze and characterize the microbes present in the sample of wastewater.
II. To study the role of isolated microorganisms from waste water (Durgapur Industrial Area) in waste degradation and also to study its role of the microorganisms from Durgapur industrial wastewater in COD reduction.
III. The Durgapur Industrial waste water is known to have lots of phenolics, cyanides and heavy metals as contaminants. Therefore our next objective was to check the damage caused to the synthetic DNA by the industrial waste water.

Characteristics of Durgapur industrial waste water:

Durgapur is one of the important industrial cities of Eastern India. There are eight large industries and more than seventy small industries in and around Durgapur. All these industries directly or indirectly let their effluents flow into the Tamla Nalah which is the only effluent carrying channel in the area. This channel originates in the asansol industrial complex as a drain for carrying rain water as well as for carrying away industrial waste effluents and flows through Raniganj, Ukhra and bifurcates at Waria; the main channel passes through Durgapur and finally flows into Damodar River, 2 Km downstream from Durgapur Barrage. The other branch called Singaran Nalah flows into the Damodar, upstream, before the barrage reservoir; the whole area provides a network of industries and agricultural fields side by side. On both sides of Tamla Nalah there are agricultural fields local people use the water of Tamla Nalah for irrigating these fields. The agricultural products are sold in different markets of Durgapur for consumption. In Durgapur, A large modern township with all amenities has grown with industries, particularly the Durgapur Steel Plant. There are around twenty thousand residential buildings along with commercial complexes, markets, hospital, and

establishments expected in any modern township. At the eastern fringe of Durgapur Steel Township, there is a sewage disposal plant operating all twenty four hours a day. For carrying sewage from the township to the disposal plant, there are two large drains passing along the two sides of the township. By the sides of these drains also there are agricultural fields and farmers of those localities use the raw sewage from these drains to irrigate their lands. These agricultural products also are marketed locally. The sewage disposal plant treats the sewage and discharges the treated water, while the Steel Plant Authority sells the soil sludge at a very cheap rate and the local farmers use this sludge as fertilizer in their agricultural fields. Thus in Durgapur, both, industrial effluents and untreated municipal sewage are used for irrigating agricultural fields while treated sludge is used as manure. From these, pollutants may be expected to in directly enter the food chain and affect human health. In past there were few incidents which shows drastic consequence of waste water pollution in the Asansol Durgapur Industrial Belt.

- 1973 Fish and frogs die by the thousands in the left bank irrigation canal of the Durgapur Barrage of the Damodar Valley Corporation. Paddy cultivation on 240 hectare of land along the canal lost.
- 1978 large number of cattle heads dies after drinking water from the canal.
- 1983 About 500 cattle heads die and 8,000 quintals of processed crop are destroyed because of contamination.

All the three disasters mentioned here were caused by the discharge of toxic chemicals containing ammonia, arsenic compounds and heavy metals into the Damodar canal water by all the small as well as large scale industries in and around Asansol Durgapur Industrial Belt. However, the only source of drawing water is river Damodar which receives industrial pollutants through two storm water drains – Nunia Nalah in the Asansol region and Tamla Nalah in the Durgapur region. So the toxic chemicals are scattered in the sediments in and around Asansol Durgapur Industrial Belt. The thermal power plants contribute the largest about 92% waste water discharge mainly which come from Durgapur Power Station. Fly ash constitutes the main pollutants. It has substantial metallic toxic load and negligible non-metallic toxic load and BOD (CPCB, 1992). The river Damodar also receives mine water discharge in the range of 0.2 to 0.5 million m3/day.(Jones GJ et al 1977)The Damodar River, Tamla Nalah, Ponds, Groundwater and various industrial units were monitored and river water quality was found within the stipulated limits in context to the general parameters and also for the heavy metals. The Tamla Nalah, which is the sink of all discharge, was having the COD in the range of 40-80 mg/l; BOD 11-25 mg/l. sediment of the river in the downstream of confluence of Tamla Nalah was having maximum iron content of about 21,000 mg/kg, zinc 45 mg/kg, copper 16 mg/kg, total chromium 28 mg/kg, nickel 17 mg/kg and lead 5 mg/kg, whereas in Tamla Nalah sediment having maximum iron content of about 22,000 mg/kg, zinc 121 mg/kg, copper 24 mg/kg, total chromium 18 mg/kg, nickel 14 mg/kg and lead as 33 mg/kg.(Homada et al 1987)Various types of toxic substances discharged as industrial effluents contaminate the water bodies. It is known that iron and steel industries may include gasification products such as Benzene, Napthalene, Anthracene, Cyanide, Ammonia, Phenol, Cresol together with a range of more complex organic compounds known collectively as Polyaromatic Hydrocarbons (PAH), Hydraulic oils and particulate solids. To protect public health environment it is necessary to have knowledge of constituents of concerns in waste water, impact of these constituents when waste water is dispersed into the environment, the transformation and the long term fate of these constituents in treatment processes, treatment method that can be used to remove or modify the constituents found in waste water, method for beneficial use for disposal of solids generated by treatment system (Pokhrel *et al*., 2004)Many industries use physical and chemical methods for the treatment of waste effluent. Nowadays advanced chemical oxidation processes are used to reduce COD and BOD levels, and to remove both organic and oxidize able inorganic compounds, but these are not that much efficient because of cost as well as environmental effects. So microbe based biological methods continue to be the prime choice for efficient and sustainable waste water processing.

Bioremediation

Bioremediation refers to the process of using microorganisms to remove the environmental pollutants i.e. the toxic wastes found in soil, water, air etc. the microbes serve as scavengers in bioremediation. The other used for bioremediation are biotreatment, bioreclamation and biorestoration (Chaudry *et al*., 1998) Interest in the microbial biodegradation of pollutants has intensified in recent years as mankind strives to find sustainable ways to clean up contaminated environments. These bioremediation and biotransformation methods endeavour to harness the astonishing, naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls, Polyaromatic carbon, pharmaceutical substances, radionuclides, metals. Major methodological breakthroughs in recent years have enabled detailed genomic, metagenomic, proteomic, bioinformatics and other high throughput analysis of environmentally relevant microorganism providing unprecedented insights into key biodegradative pathways and the ability of organisms to adopt to changing environmental conditions(Lazarova V et al 1994).The elimination of a wide range of pollutants and wastes from the environment is an absolute requirement to promote a sustainable development of our society with low environmental impact. Biological processes play a major role in the removal of contaminants and they take advantage of the astonishing catabolic versatility of microorganism to degrade/convert such compounds. New methodological breakthroughs in sequencing, genomics, proteomics, bioinformatics and imaging are producing vast amounts of information. In the field of Environmental Microbiology, genome based global studies open a new era providing unprecedented *in silico* views of metabolic and regulatory networks, as well as clues to the evolution of degradation pathways and to the molecular adaptation

strategies to changing environmental conditions (Hascoet MC *et al.,* 1985). These studies and approaches are increasing our knowledge and hence they will certainly accelerate the development of bioremediation technologies and biotransformation processes. Traditional molecular analyses have led to great understanding of the microbial diversity in natural systems. The approaches can tell the presence of a particular group of bacteria, but does not address the activity. Molecular methods, including micro autoradiography, mRNA analysis, growth assays, and incorporation of stable isotopes, can be used to determine which bacteria are involved in biodegradation of chemical pollutants. This information leads to a greater understanding of the role of microbial community structure and function with respect to bioremediation (Kampbell *et al*., 1996).Bacterial pathways for the degradation of organic pollution have been the subject of intense study for decades. However, important physiological events that precede the catabolism of these compounds have recently been receiving significant scientific attention. Bioavailability or the amount of a substance that is physiochemically accessible to microorganisms is a key factor in the efficient biodegradation of pollutants (Parekh et al 1994). Chemotaxis or the directed movement of motile organisms towards or away from chemicals in the environment is an important physiological response that may contribute to effective catabolism of molecules in the environment. In addition, mechanisms for the intracellular accumulation of aromatic molecules via various transport mechanisms are also important (Munnecke *et al*., 1976).Natural attenuation is one of several costs – saving options for the treatment of polluted environment, in which microorganisms contribute to pollutant degradation. For risk assessments and endpoint forecasting, natural attenuation sites should be carefully monitored. When site assessments require rapid removal of pollutants, bioremediation, categorized into biostimulation and bioaugmentation, can be applied. In such case, special attention should be paid to its influences on indigenous biota and the dispersal and outbreak of inoculated organisms. Recent advances in microbial ecology have provided molecular technologies, e.g., detection of degradative genes, community fingerprinting and metagenomics, which are applicable to the analysis and monitoring of indigenous and inoculated microorganisms in polluted sites (Rintala *et al*., 1991). Scientists have started to use some of these technologies for the assessment of natural attention and bioremediation in order to increase their effectiveness and reliability.

MATERIALS AND REAGENTS

1)Collection of wastewater: 2-liter cans.

2)Characterization and Sub-Culturing Of Microorganisms: Sterilized Petri plates, nutrient agar, inoculation loop, weighing balance, double distilled water. Autoclaved test-tubes, nutrient broth, 250 ml flasks, 500 ml flasks, beakers, pipette, dropper, slides.

Wastewater sample, alcohol, grams iodine, safranin dye, crystal violet dye, sodium chloride, tryptone, peptone, dipotassium hydrogen phosphate, dextrose, methyl red, kovacs reagent, ammonium dihydrogen phosphate, di-potassium hydrogen phosphate, trisodium citrate, magnesium sulphate, bromothymol blue, 2-naphthol, potassium hydroxide, isopropyl alcohol, hydrogen peroxide(Pratibha Singh et al ,Jan-2004).

3) Cod Reduction Determination:

a) Open reflux method:

500-millilitre (ml) Erlenmeyer flask , friedrichs reflux condensers,electric hot plate or six-unit heating shelf ,volumetric pipettes (10, 25, and 50ml capacity) ,burette(50 ml - 0.1 ml accuracy),burette stand and clamp,analytical balance(accuracy 0.001gram (g)),spatula,volumetric flasks (1000ml capacity), boiling beads, glass magnetic stirrer and stirring bars,ultracentrifuge Potassium dichromate ($K_2Cr_2O_7$) 0.25N, sulphuric acid (H_2SO_4),silver sulphate (Ag_2SO_4) solution,mercuric sulphate ($HgSO_4$) crystals,ferrous ammonium sulphate (FAS) [$Fe(NH_4)_2(SO_4)_2$](0.01N), ferroin indicator. (Maiti SK *et al*., 2004)

b) Using Lovibond (Germany) COD Analyzer. Digester, analytical kit, standard COD samples of range 0-1500ppm, beakers, vials, ultracentrifuge.

Methods

Collection Of Wastewater: The sample wastewater was collected from the three different points in the Asansol Durgapur industrial belt in a 2-litre cans. It was done in order to do a comparative study.

a) Waste water from Asansol industrial and domestic effluent channel. This sample is referred as GB water in this project work.
b) Waste water from Durgapur Industrial Zone it is being referred as DIW water and
c) Waste water from the main effluent carrying channels i.e. Tamla Nalah in Durgapur. The bacterial strain obtained from this water is designated as PTB strain (Clair N Sawyer -2003).

After collection of samples it had gone through various types of microbial techniques for isolating the microbial colony and to see the types of microbes present in that type of waste water sample.

A) Physical characteristics of Durgapur Industrial Waste Water: Two parameters have been checked of the waste water namely:

* PH test- It was done simply by the use of pH meter and readings of distilled water and waste water was compared.
* Conductance test- Electrical conductance, which is the inverse of electrical resistance, is a measure of how easily current can flow. The higher the conductance, the more easily current can flow. Conductance is very useful when testing water purity

B) **Isolated Bacterial growth curve** :

Bacterial growth is the division of one bacterium into two daughter cells in a process called binary fission. Providing no mutational event occurs the resulting daughter cells are genetically identical to the original cell. However, if the number surviving exceeds unity on average, the bacterial population undergoes exponential growth. The measurement of an exponential bacterial growth curve in batch culture was traditionally a part of the training of all microbiologists; the basic means requires bacterial enumeration (cell counting) by direct and individual (microscopic, flow cytometry direct and bulk (biomass),

indirect and individual (colony counting), or indirect and bulk (most probable number, turbidity, nutrient uptake) methods (Curds CR *et al*., 1970).

Bacterial growth in batch culture can be modeled with four different phases: lag phase (A), exponential or log phase (B), stationary phase (C), and death phase (D).

1) During lag phase, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs.
2) Exponential phase (sometimes called the log phase) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period.
3) During stationary phase, the growth rate slows as a result of nutrient depletion and accumulation of toxic products. This phase is reached as the bacteria begin to exhaust the resources that are available to them. This phase is a constant value as the rate of bacterial growth is equal to the rate of bacterial death (Dick *et al*., 1995).
4) At death phase, bacteria run out of nutrients and die. Fig-3

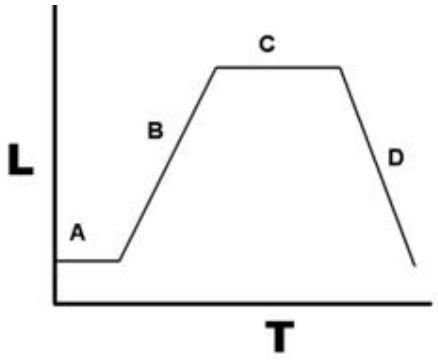

Fig-3

Growth is shown as L = log(numbers) where numbers is the number of colony, versus T (time.).

To achieve the growth pattern optical density was checked with specific time interval.

c) **Isolation of microorganisms from waste water and maintenance in solid and liquid media:**

After that serial dilution was done for the isolation of microorganisms from respective water sources.

Serial dilution

The technique used to make a single dilution is repeated sequentially using a more and more dilute solution as the "stock" solution. At each step 1ml of the previous dilution is added to 9ml of distilled water. Each step results in a 10 fold change in the concentration from the previous concentration using serial dilution method we have done 10^6 (9ml water: 1ml sample) times dilution for three types of waste water sample. In the reverse is a diagram of how waste water was serially diluted in various test tubes (Feng *et al*., 1997). Fig-4

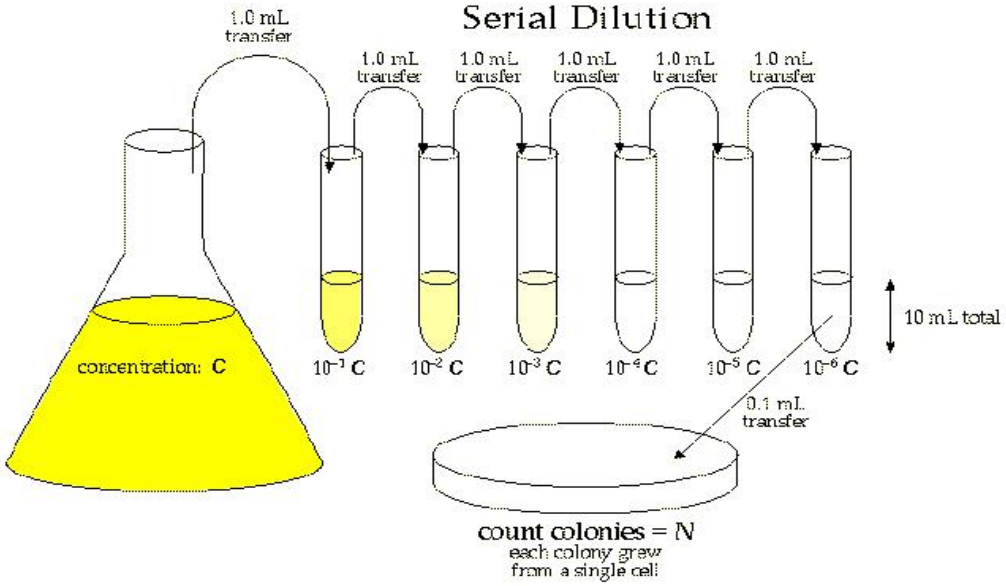

Fig-4

Preparation of media

i. Preparation of liquid nutrient media: The percentages of the ingredients that make the liquid media in 100ml solution are as follows:-
   a. Yeast extract – 1%
   b. Peptone – 1%
   c. Sodium chloride – 0.5%
   d. Water as required to make up the volume.

ii. Preparation of nutrient agar (solid) media:

The percentages of the ingredients that make the solid media in 100ml solution are as follows:-

a. Agar – 2%
b. Peptone – 1%
c. Sodium chloride – 0.5%
d. Beef extract-0.5%
e. Water as required to make up volume.

Inoculation was done with the sample waste water into Petri plates containing the above agar media and allowed the media containing sample for 48 hours incubation at 37^0c temperature. Then sub culturing of the colonies formed on the agar plates were done into test tubes containing liquid media. The sub culturing technique is mentioned below.

D) **Sub culturing microorganism:**

In the laboratory, microorganisms are usually grown or cultured in liquid (Broth) or on solid (Agar) media. Growth of bacterial and yeasts shows as turbidity in the broth although sometimes bacteria grow as a layer on the surface of the broth or at the bottom of the culture tube. Both the sub cultured Petri plates and the liquid culture medium were kept at 37 ^{o}C in an incubation chamber for 24 hrs. Growth was observed in these and was kept at 4 ^{o}C for making growth static (Lechevallier MW et al, 2004).

Biochemical tests:

1) Grams staining: First a bacterial smear is heat fixed to a microscope slide sterilized by wiping it with alcohol(A smear is a sample of bacteria suspended in a small amount of water on a slide. That sample is then dried using heat.) The heat kills the bacteria and attaches the sample to the slide so that it does not easily wash away.

The Gram staining procedure was as follows:

1. The slide was flooded with Crystal Violet (the *primary stain*).
2. After 1 minute, the slide was rinsed with water.
3. Next the slide was flooded with Iodine (Iodine is a *mordant* that binds with Crystal violet and is then unable to exit the Gram+ peptidoglycan cell wall.)
4. After 1 minute, again the slide was rinsed with water.
5. The slide was flooded with Alcohol. (Alcohol is a *decolorizer* that will remove the stain from the Gram-negative cells.)
6. After 10 or 15 seconds, the slide was rinsed with water. (The decolorizer may remove stain from the Gram-positive cells as well.)
7. Again the slide was flooded with Safranin (the *counterstain*).
8. After 1 minute, the slide was rinsed with water.
9. The slide was dried using blotting paper. It was now ready to be viewed under 40X optical microscope.
10. After this staining procedure, the Gram + cells will appear purple, having retained the primary stain. The Gram – cells will appear pink, having retained the counter stain after the primary stain was removed by the decolorizer.

2)**. Indole test**

1. 2.5 grams of tryptone and 2 grams of sodium chloride were weighed and added to 250 ml distilled water to prepare indole broth media. The media was autoclaved.
2. The tryptophan (or peptone) broth was taken in 3 separate test tubes and was inoculated with the organisms in sub cultured Petri plates of different microorganisms isolated from different water sources and incubated at 37°C for 24-28 hours.
3. On the very next day, 0.5mL of the Kovac's reagent added and gently agitated. The upper layer of the tube broth was observed for any characteristic color (French et al, 1998).

3)**. Methyl-red and Vages - Proskauer test**:

Methyl red-VP media preparation and citrate media preparation and inoculation using sub cultured organisms were done. These sub cultured organisms were again sub cultured on 3 fresh Petri plates to keep log culture of microorganisms.

1. 1.75 gm of buffered peptone, 1.25 gm of dipotassium hydrogen phosphate, 1.25 gm of dextrose was added to 250 ml of distilled water to form MR-VP broth for both methyl-red and VAGES-PROSKAUER tests. The media was autoclaved.
2. These were taken in 6 separate test tubes, 3 for methyl red and 3 for VP test.
3. First 3 test tubes were inoculated with the organisms from the 3 sub cultured Petri plates and kept for incubation for 48 hrs at 35°C.
4. Second set of test tubes were inoculated in the same way and kept for incubation for 24 hrs at 35°C.
5. Meanwhile sub culturing of the 3 Petri plates was done again to maintain the cultured microbes.
6. VP test was performed by adding 15 drops of Barrett's solution A and the test is shaken from side to side and then 5 drops of Barrett's solution B is added and again shaken vigorously for 20 minutes.
7. MR test tubes were taken and methyl red reagents were added dropwise. Color change was observed. Methyl red reagent was prepared by adding 0.02 gm of methyl red, 47 ml of ethyl alcohol in 53 ml of distilled water to make a 100 ml MR reagent.(Livernoche et al 1983)

4). Citrate test: Preparation of 250 ml of citrate media:

1. Added 0.25 gm of ammonium dihydrogen phosphate,0.25 gm of di-potassium hydrogen phosphate,1.25 gm of sodium chloride,0.5 gm of trisodium citrate,0.05 gm of magnesium sulphate,0.2 gm of bromothymol blue,3.25 gm of agar in 250 ml of distilled water. The media was autoclaved.
2. 3 slant agar test tubes were made under laminar air flow and organisms from sub cultured plates were used to inoculate these.
3. These were incubated at 37°C for 48 hrs.
4. Any characteristic color development was observed

5)**. Catalase test**:

Catalase test was done to determine presence of any catalase producing microbe.

1. 5ml of hydrogen peroxide solution was taken in 3 test tubes.
2. And then 1ml of bacterial culture is taken from the inoculated nutrient broth and added to it.
3. Any vigorous bubbling pattern was observed.

6)**. Fungal growth pattern:**

Determining any fungal presence in sample wastewater was carried out.

1. PDA media for fungal growth was prepared to see presence of any fungus in the sample wastewaters.

2. 7.8 gm of PDA media was dissolved in 200 ml of distilled water and autoclaved.
3. The media was taken in 3 separate test-tubes and inoculated with the sample waste waters.
4. The test tubes were incubated at 25-30°C for 6 days.

Effect of the industrial effluents on synthetic DNA:

100 microgram per ml synthetic DNA was taken in a test tube and 5 ml of industrial waste water was added, and kept for 10 to 90 minutes. At each time point, the O.D at 260 nm was checked and compared with the respective controls, i.e. only DNA (untreated) of 100 micro liters per ml in 5 ml distilled water and RNA (100 micro liters per ml).

E). COD: - Open reflux technique:

Chemical Preparation:

1) Sulphuric acid reagent:It was prepared by adding 5.6 gm silver sulphate in 500 ml conc. Sulphuric acid.
2) Potassium dichromate solution: 0.0147 M potassium dichromate solution was prepared by dissolving 12.259 gm of it in 1000 ml distilled water.
3) Ferrous ammonium sulphate solution : This solution was prepared just before titration in all cases. It was prepared by dissolving 98 gram ferrous ammonium sulphate in distilled water ,adding 20 ml conc.sulphuric acid,cooling and diluting to 1000 ml so that the solution becomes of the strength of 0.25 M.(Harper et al 1998)

Procedure

1. 50ml sample or an aliquot diluted to 50ml was taken in a 500ml refluxing flask. The blank was prepared using 50ml of distilled water. This is a precise measurement and hence a 50ml volumetric pipette was used.
2. 5 to 7 glass boiling beads were added.
3. 1.5 g of mercuric sulphate ($HgSO_4$), 5ml of concentrated sulphuric acid / silver sulphate solution were added to above 50 ml and mixed in a water basin.
4. Accurately 50ml of potassium dichromate ($K_2Cr_2O_7$) was added to above solution and mixed.
5. While mixing, an additional 70ml of concentrated sulphuric acid reagent was added.
6. After thorough mixing, the flasks were attached to the reflux condenser,heat applied , and refluxed for 3 hours. A reagent blank containing 50ml of distilled water and an untreated water sample were treated and put on boiling as 2 sets were being operated simultaneously.
7. In the second set on tenth day, 2 more sets of 1hr and 3 hr treatment were put on reflux apparatus. Similarly 4 hr treatment was put on eleventh day. Below mentioned procedure was applied to each one of the above samples.
8. The apparatus was cooled to room temperature after the refluxing period.
9. Accurately 10 ml of each sample was taken.
10. 4-5 drops of ferroin indicator was added to each of these samples on eleventh day and titration was done using FAS, end-point observation was made in each case. It changes from greenish yellow to red.

COD kit method:

1. Four vials were taken and filled with untreated water, distilled water, and 2 wastewaters treatments of 3 and 6 hrs. Treatment of process was repeated for all the collected waste water samples.
2. Inoculums along with broth was taken in treatment ones which were then centrifuged and pellet was taken out of them. Pellet was dissolved in wastewater for treatment samples for a period of 24 and 48 hrs respectively (Harrison WG et al, 1978).
3. The above treated samples were then centrifuged and supernatant was taken for further analysis.
4. The sample was diluted to 1:20 dilution in both cases of treated as well as untreated sample.
5. . These 4 vials were then digested in a digester for 2 hrs one by one.
6. . Subsequent analysis for COD was made using analyzer kit with distilled water as blank having 0 COD.

RESULTS AND DISCUSSION

1) Sample wastewater characteristics (Durgapur Industrial Waste Water):

Physical: a. Color: Light brown.
b. pH: (7.5+0.2) to (7.5-0.2).
c. odour: noxious smell.
d. conductivity: 7.77 S/m

Chemical: It is presumed to have a large amount of solids as indicated by its conductivity value. It contains large amount of phenol and cyanides.

2) Colony characteristics and gram staining

Sample water :	Colony Characteristics :	Gram staining :
DIW strain	Cream color, Round, Smooth.	Negative (Bacillus)
GB strain	Cream color, Round, Smooth.	Positive (Coccus)
PTB strain	Whitish colonies, Round in shape.	Positive (Coccus)

It is quite clear from the above table that the microorganisms isolated from different water source have distinct feature of their own and by gram staining test it seems Durgapur industrial water have bacterial strain, which is negative to the test, but in the second and third case the strains are positive to it.

3) Fungal growth pattern

After inoculation of PDA media with 100 micro liters of DIW waste water, There was no observable pattern of fungal growth as even after 7 days incubation at 37^{o}c only bacterial growth was seen and no mycelial growth were observed.

4) Growth curve of the isolated bacteria from Durgapur industrial waste water

The growth pattern seems to have four distinct phases of growth i.e. the growth curve has: Lag phase, Exponential or Log phase, Stationary phase, and Death phase.

a) During lag phase, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide.

b) Exponential phase (sometimes called the log phase) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population.
c) During stationary phase, the growth rate slows as a result of nutrient depletion and accumulation of toxic products. This phase is reached as the bacteria begin to exhaust the resources that are available to them.
d) At death phase, bacteria run out of nutrients and die. (Levin GV et al, 1972)

IMViC and Catalase test

TESTS	DIW – STRAIN	GB - STRAIN	PTB – STRAIN
INDOLE	NEGETIVE	NEGETIVE	NEGETIVE
MR	POSITIVE	POSITIVE	POSITIVE
VP	NEGETIVE	NEGETIVE	POSITIVE
CITRATE	POSITIVE	POSITIVE	NEGETIVE
CATALASE	POSITIVE	POSITIVE	POSITIVE

5) Biochemical test of the isolated microorganisms

It was observed that in the INDOLE test, all the isolated microorganisms showed negative result indicating that tryptophan was utilized as a nutrient source.
In the MR test presence of mixed acid fermenter is indicated in the DIW and GB tests whereas PTB showed a negative result.
VP tests indicate the presence of acetoin in the DIW case but in the rest it gives us a negative result.
CITRATE test indicates that the microorganisms from the water source DIW and GB can utilize citrate as carbon source but in case of PTB it gives a negative result.
CATALASE TEST shows positive result for all the isolated bacterial strain confirming the presence of Catalase enzyme because (Delayed) bubbles formation is observed.

6) Effect of waste water on synthetic DNA

The pollutant present in the waste water severely affects the flora and fauna of the ecosystem. To see the effect of the waste water on the DNA the absorbance of the DNA at 260 nm has been checked. The result is represented in the table below:

O.D taken at 260 nm : For the analysis of DNA damage caused by waste water:	Control DNA (100 microgram/ml)	Treated DNA
Water sample :		DIW
Time: in minutes.		
10 minutes	0.256	0.957
30 minutes	0.256	0.951
60 minutes	0.256	0.948
90 minutes	0.256	0.975

From the table above it is seen that waste water has some drastic effect on the synthetic DNA, as the absorbance of the DNA at 260 nm increased about 3.5 times when treated from 10 to 90 minutes compared to control. The increased treatment doesn't affect much in the denaturing of the DNA in this case. Though DNA is not getting denatured to single stranded form but rather the DNA confirmation is changing in between double stranded and single stranded form (0.948) at $O.D_{260}$ nm.

7) RESULT FOR COD

COD experiment was done by both the methods i.e. Open reflux method and COD kit technique. But the results were not satisfactory.

CONCLUSION

From the results of the experiments performed on waste water treatment by microorganisms, it can be concluded that microorganism present in the industrial effluent have the ability to survive in the very toxic environment of waste water and utilize the toxic pollutants as nutrient source for their growth. It is also assumed from the experiment that the waste water has some drastic consequence affect on the synthetic DNA, as well as leading to severely affect the flora and fauna. The drastic change in the optical density of the Deoxyribonucleic acid (DNA) is been noticed ,which perhaps can be due to the different aromatic ,toxic ,heavy metals ,phenol compounds present in the water . However one more reason can also be hypothesized, which can be due to some of the very specific compounds, such as (Dibromoacetic acid, dichloroacetic acid & trichloroacetic acid) which show a property of an Epigenesis, which can directly induce the increase in the optical density, which means addition of methyl group directly to the nucleotides. To some extent it also becomes clear that the microorganisms present in the waste water can be used to treat the effluent before its release into the environment. So the industrial effluents can be well treated and discharged to the receiving streams with low concentrations of pollutants in it, causing fewer effects on the flora and fauna. Thus, it can be said that from this laboratory scale study, industrial waste water can be treated in a better way by Bioremediation leading to less water pollution and it is also a cost effective and eco-friendly process. Finally, the available new technologies for global analysis of epigenetic alterations will provide insight into the extent and patterns of alterations between human normal and diseased tissues.

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