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Effect of Some Autochthonous Bacteria on Degradation of Sewage Wastewater and Its Uses in Crop Production

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Abstract: **The performance of designed specific bacteria isolate comprising of bacillus pumlllus pseudomonas aerruginosa and Brevibacterium spp in terms of their reduction capability and degrading activities of the bacteria were studied.**

Objectives: The aim of this study was to isolate some autochthonous bacteria from niche area sol and investigate their capability to degrade the components in the wastewater.

Materials and Methods: Fifty soil samples were collected from different sites from niche area along Oyun river in Ilorin East Local Government of Kwara State, and analyzed for the presence of Bacteria strain.

The media used in this research were nutrient broth and agar. Bacteria strains were identified by their morphological and biochemical characteristics. The reduction of sewage wastewater weight and degrading properties of pseudomona aeuraginosa. Bacillus Pumillus and brevibacterium spp were examined.

Results: The identified Bacteria strain included Brevibacterium spp (86.6%), pseudomona aeuroginosa (9.6%), and B.pumilus (3.6%). Evaluation of morphological physiological and Biochemical activity of the designed consortia was carried out against three different bacteria. Acid production tests indicated that this bacterium capable to utilize the citrate and hydrolyze casein catalase, and oxidase positive. The Bacterium growth rate ranges from 25 to 45^{o}C.

Conclusions: This study indicated that some *autochthonous Bacteria* have the potential to degrade sewage wastewater which can be used to enriched soil fertility.

Keywords: **Soil, Autochthonous Bacteria, Consortia, Niche.**

I. INTRODUCTION

Most treatment systems for domestic sewage depend on a wide array of aerobic and facultative anaerobic microorganisms, mainly bacteria that can utilize the raw organic material as a carbon and energy source during their growth and reproduction. As a result, these microorganisms decompose much of the organic fraction into simpler, less-toxic compounds; destroy pathogenic (i.e., disease-causing) microorganisms; stabilized the system by decreasing its volatility; and facilitate the recycling of water for domestic, agricultural and environmental use. Wastewater contains various types of bacteria, virus, protozoa, algae, fungi etc. Some of these are pathogens and harmful to the human and animal life, (1).

The using of *biological additives*, like bacteria and extracellular enzymes mixed with surfactants or nutrient solutions, was studied. Some biological additives have been found to degrade or dissipate septic tank scum and sludge.(2)

Some studies suggest that material degraded by additives in the tank contributes to increased loadings of biochemical oxygen demand (BOD), total suspended solids (TSS), and other contaminants in the otherwise clarified septic tank effluent.

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II. LITERATURE REVIEW

Until the last 200 years or so, the deterioration of watercourses due to organic pollution was not a serious problem because a relatively small human population lived in scattered communities and the wastes dumped into rivers could be coped with, by the natural self-purification properties. Water pollution became a severe problem with the industrialization of nations, coupled with the rapid acceleration in population growth. Industrialization led to urbanization, with people leaving the land to work in the new factories.

The term sewage refers to the wastewater produced by a community which may originate from three different sources: (a) domestic wastewater, (b) industrial wastewater, and (c) rain water.

Domestic wastes from the rapidly expanding towns and wastes from industrial processes were all poured untreated into the nearest rivers. Effluent waters, which should be removed from settlements and industrial enterprises, are known as sewage. Effluents are classified by their origin as domestic or public sewage, industrial effluents, and atmospheric (rain) run off. The sanitary requirements for the composition and properties of water bodies appreciably limit the discharge of sewage into water bodies (3)

The term “sewage sludge” or “biosolids” represents the insoluble residue produced during wastewater treatment and subsequent sludge stabilization procedures, such as aerobic or anaerobic digestion (3)

Very rarely do urban sewage systems transport only domestic sewage to treatment plants; industrial effluents and storm-water runoff from roads and other paved areas are frequently discharged into sewers. Thus sewage sludge will contain, in addition to organic waste material, traces of many pollutants used in our modern society. Some of these substances can be pytotoxic and some toxic to humans and/or animals (4).

Sewage sludge also contains pathogenic bacteria, viruses and protozoa along with other parasitic helminths which can give rise to potential hazards to the health of humans, animals and plants.

Sewage sludge is a bye products of the wastewater treatment process that are useful also known as Biosolid it comes from: (a)Domestic waste water (b) Industrial effluents (c) Storm water run off from roads and paved areas.

Sewage waste-water contains pathogenic bacteria, viruses and protozoa that give rise to potential hazards to health of humans, animals and plants. It is necessary to contro/l the concentration of sewage sludge in the soil and water body.

Using some biological measure in treating the sewage waste -water, some Bacteria strain isolated from the soil is necessary if organic matter is to be removed from water. Nonetheless, Biological treatment offers an economical advantage to physical and chemical treatment methods. Biosolids can be useful **for agricultural use** is a more efficient and sustainable alternative to inorganic fertilizers and mineral fertilisers - such as phosphate and/or soil conditioner to assist with the growth of animal/ crop production and to help improve and maintain the structure of the soil. Biosolids contains range of valuable nutrients that are essential for plant growth **land reclamation** provides a source of slow-release nitrogen ideal for use in land restoration. The biosolids product, often compost, is used for soil conditioning rather than as a replacement fertilizer improve the manageability, water retention, and tilth of troublesome soils Biosolids are rich in energy. Unprocessed biosolids have the heat value of a low-grade coal. Biogas from anaerobic digestion, which is approximately 60% methane, can be cleaned to create a biomethane products with an equivalent heat value of natural gas. Oil from experimental treated biosolids is also useful in oil and cement production.

Efforts to “market” biosolids generally refer to the sale of large amounts to commercial consumers. Biosolids also may be sold in bulk and in smaller quantities to homeowners and gardeners. They could be used as an alternative to commercial fertilizers and soil conditioners.

Untreated sewage from failed conventional septic systems or sewage discharged directly into the environment can percolate into groundwater, contaminating drinking-water wells with pathogens. The discharge of untreated sewage to streams can spread disease through direct contact, making such streams unfit for forms of recreation that involve skin contact with the water such as swimming and boating. Disease can also spread by indirect (secondary) contact such as through contact with rodents or insects that received primary exposure and in turn harbor the pathogens. Discharged, untreated sewage also can damage the receiving streams' ability to support healthy, living communities of aquatic organisms and can contaminate fisheries.

Every time you flush your toilet or clean a paintbrush in your sink, you may be unwittingly adding toxins to fertilizer used to grow the food in your pantry. Beginning in the early 1990s millions of tons of potentially toxic sewage sludge have been applied to millions of acres of America's farmland as food crop fertilizer. Selling sewage sludge to farmers for use on cropland has been a favored government programme for disposing of the unwanted byproducts from municipal wasterwater treatment plants. However, sewage sludge is anything but the benign fertilizer the U.S. Environmental Protection Agency (EPA) says it is.

Sewage sludge includes anything that is flushed, poured, or dumped into our nation's wastewater system – a vast, toxic stew of wastes collected from countless sources – from homes to chemical industries to hospitals. The sludge being spread on our crop fields is a dangerous mix of heavy metals, industrial compounds, viruses, bacteria, drug residues and radioactive material. Hundreds of people have fallen ill after being exposed to sewage sludge fertilizer, suffering from respiratory distress, headaches, nausea, rashes, reproductive complications, cysts and tumors. (5)

Water plays an important role in the life of human beings. It is most vital and important resource of our plant. Water is being deteriorated by environmental pollution and also by some other factors [6]. In the last few decades, limitless urbanization has caused a serious pollution problem due to the disposal of sewage and industrial effluents to water bodies. Sewage is waste matter resulting from the discharge into the sewers of human excreta and waste water originating from a community and its industries [7]. It has a high content of both inorganic and organic matter, as well as high of living biota which include pathogenic, biological degradation [8] {9]. However, these methods suffer from the drawbacks of either being costly and/or might generate secondary pollutants, which shows toxicity than the parent ones (10) (11]. Accordingly, biological methods are generally preferred for being more economical and environmentally friendly)`12] (13). Bioremediation provides an alternative to chemical treatments. Bioremediation uses naturally occurring micro organisms to degrade various types of wastes. Contaminants are often potential energy sources for microorganisms.

III. MATERIALS AND METHODS

Isolation and Identification of Bacteria from Soil:

Collection of Samples:

Soil samples were collected using some clean sterile polythene bags and sterile spatula in a niche area near sewage dump sites at Oyun river within Kwara State.

Bacillus pumilus, Brevibacterium sp, and Pseudomonas aeruginosa for the treatment of sewage wastewater.

Isolation of Bacteria:

Isolation of bacteria was done by Streaking plate method. For bacterial isolation, soil extract and enrichment media were prepared. The soil extract was prepared by incubating, 0.1 mL of the supernatant of each tube containing suspension of soil and enrichment media was inoculated in nutrient agar plates by streaking at 30°C for 24 hours. The plates were examined and the suspected colonies stained by crystal violet for 1-2min. grams iodine use to flood the slide. The stain were decolourized by acetone for 2-3secs counterstain with safranin for 2min after incubation, a part of the culture medium was directly mixed with ethyl acetate (50:50) and then stirred using a magnetic stirrer for six hours. . Isolates were later biochemically characterized using the commercial kit Analytical profile index system. Confirmed colonies were kept as consortium.

Screening of Different Consortium for Bioremediation:

A consortium is a group of specific bacterial isolates which possesses the capability to degrade the components present in the wastewater. For preparation of one consortium, we have used three to four different isolates. Different consortia were formulated randomly primarily on the basis of their morphology, color, size, shape, and so forth.

Different cultures were inoculated in 25 mL of NB and incubated overnight at 32–37°C and 180–200 rpm. These primary cultures were checked by streaking on nutrient agar plates which were then incubated at 32–37°C. These primary cultures were used for subculturing. 100 μL of culture was inoculated into 100 mL of NB and incubated at 32–37°C under shaking conditions for a period of 16–18 h. The culture was harvested by centrifugation at 4°C and 7000 rpm followed by washing twice with sodium phosphate buffer (pH 6.8–7.0). The supernatant was discarded and pellets were stored for the further experiments. At the time of experiment, different pellets were re-suspended according to the 20 consortia designed and inoculated in the sample and sample flasks were kept in shaking incubator at 180–200 rpm and 32–37°C for 32–36 h. After incubation.

RESULTS:

Table 1

COD Removal Ratio

Bacteria Dose (gm/l)	COD after 14 Days (p.p.m)	COD Removal Ratio (%)
0	135	81
0.5	130.5	81
1.5	121.5	83
1	131.5	82
4	126	83

Table 2: Morphological Characteristics of the Identified Strains

	COLONY MORPHOLOGY		
Test	Bacillus pumilus	Brevibacterium sp	Pseudomonas aeruginosa
Margin	Irregular	Irregular	Irregular
Elevation	Concave	Convex	Flat
Surface	Dull	Glistening	Dull
Opacity	Opaque	Opaque	Translucent
Gram's reaction	+ve	-ve	-ve
Cell shape	Rods	Cocci/rods	Short rods
Endospore	+	-	-
Shape	Oval	+	+
Motility	+	+	-
Fluorescence	(UV)	(UV)	

Table 3 Biochemical Test of Identified Strains

Tests	Bacillus pumilus	Brevibacteriumsp.	Pseudomonas aeruginosa
Growth on MacConkey agar	−	−	+
Indole test	−	−	−
Methyl red test	+	−	+
Voges Proskauer test	−	+	−
Citrate utilization	−	−	-
Casein hydrolysis	+	−	+
Gelatin hydrolysis	−	+	−
Starch hydrolysis	-	-	−
Urea hydrolysis	−	−	−
Nitrate reduction	−	−	−
H2S production	−	−	−
Catalase test	+	+	+
Oxidase test	−	+	+
Acid production from carbohydrates			
Salicin	−	+	−
Arabinose	−	−	+
Galactose	−	+	+
Dextrose	+	−	+
Meso-Inositol	−	+	−
Raffinose	−	−	−
Rhamnose	+	−	−
Fructose	+	-	+
Mannitol	−	−	+
Sucrose	+	−	−
Xylose	−	−	+

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Table 4: Study of Physiological Features (temperature,) of Identified strains

(−) represents no growth and (+) growth

Bacillus pumilus	Brevibacterium sp	Pseudomonas	aeruginosa
Growth at 4°C	−	−	−
10°C	−	−	−
25°C	-	+	-
30°C	+	+	-
37°C	-	-	-
42°C	+	+	+
45°C	+	-	-
55°C	−	−	−
65°C	+	−	

IV. RESULT AND DISSCUSSION

Thirty eight (38) bacterial isolates were purified from all the above-mentioned isolation procedure. It was hypothesized that bacteria isolated from their natural habitat have capability of surviving in harsh conditions by developing some catabolic enzymes systems, specific for particular components present in the natural habitat. The isolated colonies were diverse in their morphologies, ranging from small pin-pointed to large sized, smooth margined to wrinkled periphery, shining to dry, and so on. After three weeks the volume of sludge and the concentration of COD in sludge and sludge mass were calculated, see table 1.

Supplementary Table 2 shows the morphological characteristics of those isolated bacterial population obtained during the process of isolation.

Strains were identified on the basis of physiological, morphological, biochemical, and 16rRNA techniques performed at the University of Ilorin Teaching Hospital (UITH) Laboratory. Strains of the selected consortium 10 were identified as Bacillus pumilus, Brevibacterium sp, and Pseudomonas aeruginosa.

The results in table 1 showed that COD concentration versus the different doses of bacteria is from 0.0 to 4.0 gm/l after retention time (14) days were much closed. The COD in reference sample nearly closed to the samples with bacteria. After three weeks the volume of sludge and the concentration of COD in sludge and sludge mass were calculated, see Table 1.

Morphological characteristics (margin, elevation, surface, opacity, gram's reaction, cell shape, endospore, position, shape, motility, and fluorescence) of the identified strains is shown in Table 2.

Physiological tests and various biochemical tests were also carried out, and the results showed that Bacillus pumilus, is aerobic in nature, gram positive, motile, shows its growth from 25 to 45°C, capable to starch hydrolysis, and catalase positive. Brevibacterium sp. (is aerobic in nature, gram positive, motile, shows its growth from 25 to 45°C catalase and oxidase positive. Pseudomonas aeruginosa is aerobic in nature, gram negative, motile, shows its growth from 25 to 42°C, this bacterium capable to utilize the citrate and hydrolyze casein, catalase, and oxidase positive (Tables 2 and 3).

Different temperatures were also studied for better COD reduction. The results reveal that better COD (chemical oxygen demand) reduction could be achieved in the flask incubated at 35°C as compared to the other flasks incubated at 25°C, 40°C, and 45°C.

V. CONCLUSION

Based on the study under the condition of the experiments which was mentioned**.** The laboratory analyses to the using bacteria strains were indicated that the microbial consortiums utilized in the research are composed by harmless, widespread, ubiquitous microorganisms with known degrading capabilities, The use of such specific consortia can overcome the inefficiencies of the conventional biological treatment facilities currently operational in sewage treatment plants.

As a result of reducing the volume of sludge in all the stages of experimental works, the use of this bacteria increased the capacity of the sludge holding unit, and it's expected that the released biosolids from the sludge will be reduced and the nutrients for soil enrichment will be enhanced Heavy metal load present in the biosolids can cause harmful effect to the soil and environments.

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