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BIODEGRADATION OF SEWAGE WASTEWATER USING SOME BACTERIA STRAIN ISOLATED FROM THE SOIL AND ITS APPLICATION IN CROP PRODUCTION

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Abstract

The performance of isolated designed consortia comprising Bacillus pumilus, Brevibacterium sp, and Pseudomonas aeruginosa for the treatment of sewage wastewater in terms of morphological characteristics, physiological features and Biochemical properties was studied with aim of optimized different parameters (inoculum size, agitation, and temperature) to achieve effective results in less period of time. The results obtained indicated that consortium in the ratio of 1 : 2 (effluent : biomass) at 200 rpm, 30°C is capable of effectively reducing the pollutional load of the sewage wastewaters, within the desired discharge limits, that is, 32 mg/L, 8 mg/L, 162 mg/L, and 190 mg/L. The use of such specific consortia can overcome the inefficiencies of the conventional biological treatment facilities currently operational in sewage treatment plants.

Key word: Consortia, Sewage wastewater, Morphological, Conventional.

Introduction

Domestic wastewater will contain both solid and dissolved pollutants including faecal matter, paper, urine, sanitary items, food residues and a variety of other contaminants. Collecting and treating the wastewater is more beneficial to human health than the health service because it stopped water-borne diseases such as cholera and typhoid. Treatment of sewage is essential to ensure that the receiving water into which the effluent is ultimately discharged is not significantly polluted. However, the degree of treatment required will vary according to the type of receiving water. Thus, a very high degree of treatment will be required if the effluent discharges to a fishery or upstream of an abstraction point for water supply. A lower level of treatment may be acceptable for discharges to coastal waters where there is rapid dilution and dispersion.

Nowadays most farmers use chemical fertilizers as source of nutrients for their crop. Chemical fertilizers produce side effects to the soil itself. Normally chemical fertilizers contain chemical substances that are harmful to the soil if they are used for long term. Biosolids has been identified as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming. The utilization of microbial products has several advantages over conventional chemicals for agricultural purposes:{a)microbial products are considered safer than many of the chemicals now in use:{b)neither toxic substances nor microbes themselves will be accumulated in the food chain;(c)self-replication of microbes circumvents the need for repeated application;(d)target organisms seldom develop resistance as in the case when chemical agents are used to eliminate the pests harmful to plant growth: (Weller, 1988; Gloud, 1990; Shen, 1997).

All sewage wastewater treated produce biosolids, which are the stabilized residuals that settle from the water during the various treatment processes. Biosolids are rich in both organic matter and essential plant nutrients and can be utilized in a variety of ways, directly as a soil amendment and fertilizer, and indirectly as a feedstock in the fabrication of value-added products. Sludges are formed in the sewage treatment plants and are the unavoidable result of treating the domestic sewage and industrial effluent. Failure to regularly remove the sludges from the sewage treatment works inevitably results in the works failing, which then have an adverse effect on the receiving watercourse.

In general terms, the agricultural sector tends to promote use of manures and compost, but to date there appears to be little information promoting use of biosolids as a complement to the traditional use of manures and fertilizers for the agricultural sector. New processes, technologies and waste management strategies mean that the wastes are more environmentally acceptable, and threats to human health and the environment have been reduced or eliminated. A number of new and ongoing projects and initiatives related to the use of biosolids have prompted the need for a review of the existing policy, legislation, best management practices and guidelines (standards, objectives) in the management and beneficial use of biosolids. These activities also include sludge from industrial processes with high organic contents.

2. 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: domestic wastewater, industrial wastewater, and 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 [Arcak. et al.2000]. 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 [Arcak. et al.2000].

Very rarely do urban sewage systems transport only domestic sewage to treatment plants; industria l effluents and storm-water runoff from roads and other paved areas are frequently discharged into s ewers. Thus sewage sludge will contain, in addition to organic waste material, traces of many pollu tants used in our modern society. Some of these substances can be pytotoxic and some toxic to hum ans and/or animals (Dean and Suess, 1985). Sewage sludge also contains pathogenic bacteria, virus es and protozoa along with other parasitic helminths which can give rise to potential hazards to the health of humans, animals and plants (WHO 1981). Sewage sludge is a bye products of the wastew ater treatment process that are useful and known as Biosolid coming from: Domestic waste water I ndustrial effluents Storm water run off from roads and paved areas. It is necessary to control the co ncentration 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 to 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 be 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 to ns 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 go vernment programme for disposing of the unwanted byproducts from municipal wasterwater treatm ent plants. However, sewage sludge is anything but the benign fertilizer the U.S. Environmental Pr otection Agency (EPA) says it is.

Sewage sludge includes anything that is flushed, poured, or dumped into our nation's wastewater s ystem – a vast, toxic stew of wastes collected from countless sources – from homes to chemical ind ustries and hospitals. The sludge being spread on our crop fields is a dangerous mix of heavy meta ls, industrial compounds, viruses, bacteria, drug residues and radioactive material. Hundreds of pe ople have fallen ill after being exposed to sewage sludge fertilizer, suffering from respiratory distre ss, headaches, nausea, rashes, reproductive complications, cysts and tumors. (CFS, 2015)

Water plays an important role in the life of human beings. It is most vital and important resource o f our plant. Water is being deteriorated by environmental pollution and also by some other factors [Moschellaet al. 2005]. 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 m atter resulting from the discharge into the sewers of human excreta and waste water originating fro m a community and its industries [Guardabassiet al. 2002]. It has a high content of both inorganic and organic matter, as well as high living biota which include pathogenic, biological degradation [T chobanoglous et al. 2003, Adav, 2007]. However, these methods suffer from the drawbacks of eith er being costly and/or might generate secondary pollutants, which shows toxicity than the parent on es (Kuriharaet al, 1990; Mazumder and Roy 2000]. Accordingly, biological methods are generally preferred for being more economical and environmentally friendly (Evans, Furlong, 2003 Sogbanm u and Otitoloju 2013). Bioremediation provides an alternative to chemical treatments. Bioremedia tion uses naturally occurring micro organisms to degrade various types of wastes. Contaminants ar e often potential energy sources for microorganisms.

3. Materials and Methods

Isolation and Identification of Bacteria from Soil

Soil samples were collected using some clean sterile polythene bags and sterile spatula in a niche a rea near sewage dump sites at Oyun river in Ilorin, Kwara State. Bacillus pumilus, Brevibacteriums p, and Pseudomonas aeruginosa for the treatment of sewage were selected wastewater. Isolation of bacteria was done by Streaking plate method. For bacterial isolation, soil extract and enrichment m edia 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 s treaking at 30°C for 24 hours. The plates were examined and the suspected colonies stained by cry stal violet for 1-2min. grams of iodine were 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 mediu m was directly mixed with ethyl acetate (50:50) and then stirred using a magnetic stirrer for six hou rs. Isolates were later biochemically characterized using the commercial kit Analytical profile inde x system. Confirmed colonies were kept as consortium.

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 fo ur different isolates. Different consortia were formulated randomly primarily on the basis of their m orphology, color, size, shape, and so forth. Different cultures were inoculated in 25 mL of NB and i ncubated overnight at 32–37°C and 180–200 rpm. These primary cultures were checked by streakin g on nutrient agar plates which were then incubated at 32–37°C. These primary cultures were used for sub culturing. 100 µL of culture was inoculated into 100 mL of NB and incubated at 32–37°C u nder 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 superna tant was discarded and pellets were stored for further experiments. At the time of experiment, diffe rent pellets were resuspended according to the 20 consortia designed and inoculated in the sample a nd sample flasks were kept in shaking incubator at 180–200 rpm and 32–37°C for 32–36 h. after in cubation. The result obtained are stated in tables 1-3.

Table 1: Morphological Characteristics of the Identified Strains

COLONY MORPHOLOGY

Test	**Bacillus pumilus**	**Brevibacterium sp**	**Pseudomonas aeruginosa**
Margin	Irregular	Irregular	Irregular

Elevation	Convex	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 2: Physiological Features (pH, temperature, and NaCl concentration) of Identified strains. (−) represents no growth and (+) growth

Test	**Bacillus pumilus**	**Brevibacterium sp**	**Pseudomonas aeruginosa**
Growth pH4^{o}C	–	–	–
pH 5.0	–	–	+
pH 6.8	+	+	+
pH 8.0	+	+	+
pH 9.0	+	–	+
pH 11.0	–	–	–
Growth at 4°C	–	–	–
10°C	–	–	–
25°C	+	+	+
30°C	+	+	+
37°C	+	+	+
42°C	+	+	+
45°C	+	+	+
55°C	–	–	–
65°C	–	–	–
Growth on NaCl (%)			
2.0	+	+	+
4.0	+	+	+
7.0	+	+	-

8.0	+	-	-
10.0			

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	–	–	+

4. Discussion of Results

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. Supplementary Table 1 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). Strains of the selected 10 consortium were identified as Bacillus pumilus, Brevibacterium sp, and Pseudomonas aeruginosa. 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 utilizes 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

5. Conclusion

The selected formulated bacterial consortium comprising of the isolated bacterial strains acts in a synergistic way and is capable of degrading the easily assimilable organic compounds present in sewage wastewater. This consortium is capable of effectively reducing the pollutional load of the sewage wastewaters. The use of such specific consortia can overcome the inefficiencies of the conventional biological treatment facilities currently operational in sewage treatment plants.

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