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Assessment of Mineral Accumulation by Lysinibacillus sphaericus from Restaurant Liquid Waste

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AMS and BJA designed the study, wrote the protocol, managed the literature searches and author AMS wrote the first draft of the manuscript, performed the analysis. Authors AMS, BJA and ATO managed the analyses of the study. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Presence of microorganisms and relatively high mineral contents mainly heavy metals in waste generated from restaurants and released into the environment may indicate a negative outcome of a microbial metabolic process with serious economic and health implications. Therefore it is justifiable to examine the minerals contents and microbial resistance/accumulation of restaurant liquid wastes with the view to controlling environmental hazard through the removal of pollutants in the waste products, hence this study. Restaurant wastewater are collected from washing dishes and waste collection tank, then mixed and analyzed for the presence and quantity of minerals contents. The physicochemical parameters such as metals, heavy metals and active ingredients of the waste were evaluated. The ability of *Lysinibacillus sphaericus* to accumulate the minerals was also examined. The results showed that the sample was composed of magnesium (54.4 \pm 0.10 mg/L), potassium (23.2 \pm 0.01 mg/L), sodium (39.2 \pm 0.02 mg/L), zinc (0.61 \pm 0.01 mg/L), copper

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 $(0.08 \pm 0.01 \text{ mg/L})$ and iron $(31.6 \pm 0.03 \text{ mg/L})$. The presence of high amount of some minerals in wastewater is an indication that the environment may be polluted if the wastewaters are released in an uncontrolled manner. Also, the ability of *Lysinibacillus sphaericus* to assimilate these suggests that the wastes offers favourable condition for microbial growth and are therefore very susceptible to bioaccumulation.

Keywords: Bioaccumulation; microorganisms; mineral contents; wastewater.

1. INTRODUCTION

Water is the most indispensable natural resource in the world for the sake of existence of life. Water quality is a critical public health concern in Nigeria. Water pollution in Nigeria has come to the alarming proportion in recent. Thus, the estimation of water quality is very important for the proper assessment of associated hazards [1]. Recently, several indigenous and useful species are dwindling day by day due to contamination of soil by wastewaters. To cope with these problems, there is a need for application of oriented research so that we can better utilize the most important resource of ours. Physicochemical characteristics in many ways have significant influence and impact on aquatic life [2]. Any alteration in these parameters may disturb the quality of water. Dissolved oxygen is of great importance to all the living organisms and is considered to be the sole parameter which to a large extent can reveal the nature of whole water body. Eutrophic water bodies have a wide range of dissolved oxygen and as such oligotrophic water bodies have a narrow range of dissolved oxygen [3]. Water pollution is generally indicated by the presence of harmful/harmless microbes and minerals. Microbial examination and presence of minerals in water is a direct measurement of deleterious effects of pollution. Microbial metal accumulation has received much attention in the last years due to the potential of microorganism for cleaning metal in polluted water [4]. However, considerably less attention has been paid to the role if microorganism for metal conversion in leachate even though the same process may occur there as it occurs in soil [5].

Chemical methods such as precipitation, oxidation or reduction have been widely used to remove metal ions from industrial wastewater. Those methods are ineffective and expensive [6]. The activity of microorganisms is extended to environmental management, and microbes have superseded the conventional techniques for remediation [7]. Biological methods such as biosorption and bioaccumulation provide a promising alternative to chemical methods.

Biological processes are typically implemented at low cost. The rate at which microorganisms accumulate contaminants is influenced by the specific contaminants present, the environmental factors such as temperature, oxygen supply, nutrient supply, pH, the availability of the contaminants to the microorganism, and the concentration of the contaminants (high concentrations may be toxic to the microorganism) [6,7].

Bioaccumulation is the uptake and removal of inorganic and organic pollutants from substances by microorganisms, with bacteria and fungi being the most common organism for reclamation, immobilization, and detoxification of metallic and radionuclide pollutants. The discharge of wastewater from municipal sewers containing inorganic and organic compounds is hazardous to human health [8]. The aim of this study was to examine and account for the presence of inorganic compounds/several physico-chemical parameters and its assimilation by resistant microbes in wastewater collected from Falegan restaurant. Assessment of wastewater quality is done to analyse the physical, chemical and biological characteristics of water [9].

2. MATERIALS AND METHODS

2.1 Study Site and Collection of Samples

Falegan restaurant is one of the biggest restaurants which is located at 57, Sectariat Road, Ado Ekiti, Ekiti State. In the present investigation, the restaurant wastewater samples were collected from two different locations at the restaurant i.e Washing dishes and Waste collection tank. The wastes were then mixed together using a modified method of [10]. The wastewater samples were taken in plastic bottles and analysed for selected physicochemical parameters such as metals, heavy metals and active ingredients [11]. The hydrogen ion potential (pH) was noted onsite, while other parameters were analysed in the laboratory after the samples were properly transported using a pre-cleaned 1 L plastic container in an icebox [12].

2.2 Source of Microorganism and Domestic Wastewater

Lysinibacillus sphaericus isolated from dietary oil-rich wastewater was characterized and identified molecularly. The isolate was collected from Microbiology Department Laboratory, Ekiti State University, Ekiti State. For this study, the Optical density of 0.5 at 550 nm which equates (10^{6} CFU/ml) was then used as the population size. Wastewater sample was collected from Falegan restaurant, Ado-Ekiti, Ekiti State. Five millilitre (5 ml) concentrated HNO₃ acid was added to 200 ml of the wastewater sample in 250ml beaker to retain the metals and heavy metals present [13]. The sample was taken to Microbiology Laboratory, Ekiti State University where further analyses were performed.

2.3 Mineral Analyses of the Restaurant Waste Water

The mineral was analysed in the wastewater mixture using both the Atomic Absorption Spectrophotometer (AAS) and Flame Photometer. Atomic Absorption An (PYE spectrophotometer Unicam Sp 9. Cambridge, UK) was used for the analysis of the following heavy metals; Lead (Pb), Copper (Cu), Manganese (Mn), Nickel (Ni), Iron (Fe), while Potassium (K), Sodium (Na), Magnesium (Mg) and Calcium (Ca) were determined using a Flame Photometer (BUCK 2010 VGP) [12].

2.4 Proximate Analysis of Domestic Waste Water

The proximate composition of the wastewater samples obtained from different collection sites was analyzed by the method of the [12]. The samples were collected at 2h intervals (from 8a.m to 4p.m and pooled together) twice weekly for four weeks. The samples were kept in an iced bag immediately after collection to retain its content and stop further degradation. Two hundred milliliter (200 mL) of the fresh wastewater was sterilized by Ultraviolet (UV) with 60Hz (Model FG-Y15W). radiation Ultraviolet (UV) radiation was used in order to retain the oil in the sample. The samples were

analyzed for fat, oil, fibre, protein, ash, carbohydrate (glucose) and moisture content. The active ingredients (silica, soda ash, surfactant and phosphate) of the detergent (Omo (B) used in dish washing, were also determined with a Spectronic 20 colorimeter (Gallenkamp, UK) as described by [12].

2.5 Determination of mechanism for bioremediation

Lysinibacillus sphaericus C3-41 was grown in the basal medium containing the domestic waste water sample and incubated for 7days at room temperature. One loop full of *Lysinibacillus sphaericus* was then inoculated into 5ml sterile distilled water and read on an absorption spectrophotometer every 24 hours [7,8]. The same procedure was conducted for 7 days. This was done in order to determine the mechanism used by the organism during the remediation process. Continual growth/no growth of the organism during the process gives a clue for the remediation method as either accumulation or sorption [14].

2.6 Bioaccumulation of Inorganic Compounds in the Experimental Samples

Lysinibacillus sphaericus C3-41 was incubated separately in the sterilized oil rich waste water medium and in combination with indigenous organisms in unsterilized oil rich waste water medium to test their accumulation abilities under non-agitated conditions. About 500ml of wastewater sample was warmed in sterile round bottom flask in a water bath (Model CS200) at 47°C and shaken thoroughly to ensure the fats and oils present become uniformly distributed and homogeneity was achieved throughout sampling [15].

About 100 ml of the oil rich waste water samples in each conical flask was used for the remediation process, *Lysinibacillus sphaericus* C3-41 was inoculated in all the samples except the sterile control such that Sample 1 contained sterile sample, and was inoculated with *Lysinibacillus sphaericus* C3-41.

Sample 2 contained non-sterile sample containing indigenous organisms, it was then inoculated with *Lysinibacillus sphaericus* C3-41 and also, Sample 3 contained the control sample which was sterile and contained no organism.

Sample 1 and sample 3 containing sterile samples were sterilized by Ultraviolet (UV) (Model 60Hz radiation with FG-Y15W). Lysinibacillus sphaericus C3-41 and the indigenous organisms were present in the nonsterile sample (Sample 2). Sample 3 contained the control. All the samples were incubated at 30°C for 5days under non-agitation condition [16].

After the accumulation process, the samples were centrifuged at 12,000 (rpm) to separate the supernatant and the residual organism. The supernatant contains the heavy metals that were left un-accumulated while the residual samples contain the organisms. The (residue) organisms were then lysed immediately so that heavy metals contents that have been accumulated over the period of days will be released out. Atomic absorption spectrometer was then used to analyze the amount of heavy metals present in both organisms and the supernatants [16].

3. RESULTS AND DISCUSSION

The most commonly occurring mineral contents of the domestic wastewater sample from Falegan restaurant, Ado-Ekiti include magnesium (54.4 ± 0.10 mg/L/ 149.9 °), potassium (23.2 ± 0.01 mg/L/ 63.9 °), sodium (39.2 ± 0.02 mg/L/ 108.1 °), zinc (0.61 \pm 0.01 mg/L) and copper (0.08 \pm 0.01 mg/L) iron (31.6 ± 0.01 mg/L) (Table 1). It was observed that Magnesium had the highest concentration (54.4 mg/L) among the metals analyzed from the domestic wastewater in this work. Meanwhile, [17] reported an amount of 39.4 mg/L Mg from the same source during the dry season. An increase in the concentration of magnesium in this work could also be due to the high intensity of rainfall that solubilized the metal because the wastewaters were collected during raining season [7]. High concentration of iron (31.6 mg/L) might result from corrosion of the metallic materials used in washing and cleaning of the restaurant utensils. Iron has tendency to dissolve whenever they come in contact with oxygen, meanwhile moisture water and dominated the waste which may be responsible for the release of iron. This report is in agreement with the study of [18] who reported relatively low concentrations of some heavy metal ions (e.g. Co²⁺, Cu²⁺, Zn²⁺ and Ni²⁺) essential for microorganisms since they provide vital co-factors for growth. The incidence of high concentration of sodium (Na) and potassium (K) in wastewater can be attributed to a large amount of alkaline cleaners and detergents used in cleaning the kitchen utensils. This observation conforms to the report of [19]. The analysis of the active ingredients in the domestic waste water (Table 2) revealed that phosphate had the highest concentration of 6.41mg/L. It was also observed that sulphate contributed an appreciable amount, 4.67mg/L to the ingredients. Phosphorous is most commonly found in the form of inorganic phosphates which usually originates from agricultural run-off and from household detergents [20]. Both silica and soda ash were detected at low concentrations, 1.68 mg/L and 0.82 mg/L respectively. Similarly, the amount of surfactant extracted was very low (0.02mg/L). Many of these compounds are harmless at relatively low concentrations [20].

From this work, organic compounds consist primarily of carbohydrates (31.3%), proteins (63%) and fats (5.8%), which reflect the diet of the people (Table 3). Chemically, wastewater is composed of organic (70%) and inorganic (30%) compounds as well as various gases [21]. In food industry wastewaters, oil, proteins, carbohydrates, nutrients and suspended solids are in high concentrations.

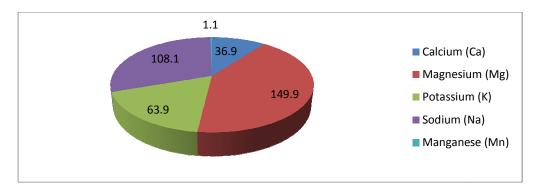


Fig.1. Mineral components (mg/L) of domestic waste water

Heavy metals	s Concentration
Copper	0.08 ± 0.00
Iron (Fe)	31.6 ± 0.03
Lead (Pb)	ND
Nickel (Ni)	ND
Zinc (Zn)	0.61 ±0.01
	ND: Not detected

Table 1. Heavy metal contents (mg/L) of domestic waste water

Table 2. Non metal and active components (mg/L) of domestic waste water

Active ingredients	Concentration
Phosphate (PO ₄)	6.41 ± 0.01
Sulphate (SO ₄)	4.67 ± 0.01
Silica	1.68 ± 0.02
Soda ash	0.82 ± 0.01
Surfactant	0.02 ± 0.00

3.1 Bioaccumulation of Inorganic Compounds by *Lysinibacillus sphaericus* C3-41

Among the inorganic ions found in the sample, Lysinibacillus sphaericus C3-41 was found to accumulate about 17.8 mg/L of potassium and 13.1mg/L of calcium, resulting in about 76.7% and 97.7% of each component absorbed respectively by the organism. It was observed that Lysinibacillus sphaericus accumulated 4.19 mg/L of phosphate present in the sterile sample amounting to about 65.4%. Wani et al., [22] reported 64.7% accumulation by Pseudomonas and Bacillus. other bacteria reported as Psolubilizers include Rhodococcus, Arthrobacter, Serratia, Chryseobacterium sp. [21], Azotobacter [23], Enterobacter, Pantoea, and Klebsiella [24], Vibrio proteolyticus, Xanthobacter agilis [25]. This investigation confirmed the capability of Lysinibacillus sphaericus in the solubilisation and accumulation of phosphate salts. This might also be due to the presence of organic acids such as fatty acids released by the organism which aid the salt dissolution. This is in agreement with [25] who reported that organic acid has been suggested as the principal mechanism of Phosphate solubilization. Also, polysaccharides (organic acids) possess some roles in the microbial mediated solubilization of Phosphate because they reduce the pH of substances as conducted [26]. Phosphate solubilizing bacteria normally produced a significant amount of EPS

and demonstrated a strong ability for Psolubilization. However further studies are necessary to understand the relationship between EPS production and phosphate solubilisation [27,28]. In actively decomposing waste the temperature rises and the pH falls rapidly and many metal ions which are relatively insoluble at neutral pH can become dissolving in the waste [27]. There was reduction in minerals such as potassium (23.2 mg/L), sodium (39.2 mg/L), zinc (0.61 mg/L) and copper (0.08 mg/L) in oil (extracted from wastewater) which might have been brought about by heat during cooking or accumulation by the organism. Although at low concentration, 75% of copper was utilized within five days by L. sphaericus. In some instances, metals have been precipitated with some metal hydroxides or undergone adsorption to the extracellular layer of microbial cell [19]. [29] reported that hydroxides of lead Pb(OH)₂ Nickel Ni(OH)₂ Cobalt Co(OH)₂ and Silver Aq(OH)₂ can easily precipitate whenever sulfate is present in high pH [8-11] forming PbS_NiS_CoS and Ag₂S. Interestingly, there were large amounts of sulphate (4.67mg/L) in the wastewater sample. However, in this wastewater, about 0.06 mg/L of copper and 0.02 mg/L of zinc were accumulated by the organism resulting into 75% and 33.3% loss of these components in the sample respectively. In most of the minerals, there was no considerable change and variations in the amount of the minerals accumulated when Lysinibacillus sphaericus C3-41 was used singly and in combinations with the indigenous organisms. The utilization of most of these minerals might be explained through the metabolic process of the organism which may be using them as substrate or co-factors. [30] reported that assimilation of Mg⁺² significantly stimulate enzyme production. However, it was not the same in the case of potassium in Lysinibacillus sphaericus which C3-41 accumulated about 76.7% and when in combinations, the amount accumulated reduced to 18%.

This considerable amount of potassium utilized may be due to the fact that few microbes release some minerals into the environment during export. This is in agreement with the work [18] who reported that amount of minerals and organic matters used up by the microbial cells of *Pseudomonas* sp, *Klebsiella* sp, *Staphylococcus* sp, and *Bacillus* sp was similar to the quantity released into the environment.

Sampling period (week)	Proximate content						
	Fat	Oil	Carbohydrate	Crude protein	Fibre	Ash	Moisture content (%)
1	$2.00 \pm 010^{\circ}$	1.85 ± 0.04 ^a	1.01 ± 0.10 ^d	1.37 ± 0.01 ^b	4.72 ± 0.01^{a}	0.82 ± 0.03^{a}	85.20 ± 0.10 ^a
2	1.99 ± 010 ^b	1.96 ± 0.01 ^{ab}	1.04 ± 1.00 ^c	2.02 ± 0.10^{bc}	5.77 ± 0.04 ^a	1.11 ± 0.00 ^a	84.31 ± 0.10 ^a
3	1.96 ± 010 ^b	1.93 ± 0.01 ^a	1.02 ± 0.10 ^d	2.02 ± 0.01^{bc}	4.10 ± 0.04^{d}	1.18 ± 0.00 ^a	88.33 ± 0.10 ^a
4	1.99 ± 010 ^c	1.93 ± 0.01 ^a	1.04 ± 0.10 ^a	2.03 ± 0.10^{bc}	6.40 ± 0.20^{d}	$1.40 \pm 0.00^{\circ}$	87.20 ± 0.10 ^d

Table 3. Proximate component (mg/mL) of domestic wastewater sample

All data were mean and standard deviation of triplicate determinations *

Table 4. Bioaccumulation of inorganic components (mg/L) in the domestic waste water

			Sterile	Non sterile		
		Control	Amount assimilated	Amount left in the sample	Amount assimilated	Amount left in the sample
Metals	K	23.2	17.8	1.30	4.19	1.10
	Mg	54.4	32.0	19.1	49.9	27.1
	Ca	13.4	13.1	ND	13.4	ND
	Na	39.2	25.8	8.9	35.0	7.9
	Fe	31.6	14.3	14.6	14.1	19.9
	Cu	0.08	0.01	0.06	0.06	0.02
	Zn	0.06	0.00	0.06	0.02	0.03
Non metals	Mn	0.40	0.00	0.40	0.00	0.40
	PO₄	6.41	4.19	1.00	4.99	4.61
	SO ₄	4.67	ND	4.67	1.17	1.40
			 Sample obt 	tained after centrifugation and treat	tment	

Sample obtained after centrifugation and treatment

Keys: ND: Not Detected

Sterile: Sample to which Lysinibacillus sp. was incorporated after sterilization Non sterile: Sample to which Lysinibacillus sp was incorporated without sterilization. Amount Assimilated: Amount of inorganic component accumulated by the organism Amount left in the sample: Amount of inorganic component left in the sample

4. CONCLUSION

Lysinibacillus sphaericus C3-41 has also been studied for their special characteristics applicability in various bio-processes such as accumulation of heavy metal/metals including Fe^{2+} , Mg^{2+} , Na^+ and assimilation of degraded minerals. It is evident from this work that *Lysinibacillus sphaericus* C3-41 has potential roles in the bioremediation of heavy metals which might contaminate and be deleterious to the aquatic environment.

5. RECOMMENDATION

I recommend that the utensils utilized should also be sanitized before and after the usage. Restaurant owners should endeavor to keep their wastewater clean before pouring into the environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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