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ASSESSING THE EFFECTS OF MICROBIAL DIVERSITY ON INDUSTRIAL EFFLUENT TREATMENT IN CHALAWA, KUMBOTSO, KANO STATE, NIGERIA

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ABSTRACT

The rapid growth of urbanization and industrialization for economic expansion has had a negative impact on biological diversity, which is one of the primary concerns of emerging countries. Microbes play an important role in polluted site decontamination and reducing the pollutant load of textile effluent. The current study focused on evaluating the effects of microbial diversity on industries and wastewater treatment in the Chalawa Industrial Area. Water samples from industrial and non-industrial locations, as well as effluent samples from before and after treatment, were studied, and it was discovered that microbial diversity was higher in river water at the industrial site (Chalawa) than at the non-industrial site (Tamburawa). Similarly, untreated sewage has higher microbial populations than treated effluent from standard treatment systems. However, the following microbes were identified: *Pseudomonas sp., Achromobacter sp*. (bacterial species), and *Aspergillus fumigates* (fungal species), which were detected solely at the industrial site and have been shown to have dye effluent decolorization capacity. A comparison of distinct microbial communities from various dye wastewater sources and textile effluents was conducted, revealing that bacteria breakdown dyestuffs and lower wastewater toxicity, among other things. The study concludes that microbial consortia serve to monitor contaminants and reduce their impact.

Keywords: Industrial effluent, Microbial diversity, Wastewater treatment

INTRODUCTION

Microbial diversity is the most amazing reservoir of life in the biosphere, which we have only begun to investigate and comprehend. Microbes have evolved to adapt to incredibly diverse settings over millennia, resulting in the development of a vast array of novel metabolic pathways or catabolic enzyme libraries. Men have long used this metabolic riches in activities such as fermentation, antibiotic and vitamin synthesis. They are also used to assess the quality of water bodies through the quantitative and qualitative presence of bacteria. Draining wastewater into a water body boosts nutrient levels, promoting microbial development capable of degrading and using xenobiotic and refractory chemicals for energy. This initiates a complicated alteration in microbial diversity (Jain et al., 2005). The textile sector is one of the most significant and powerful contributors of high COD, color, and organic matter in wastewater (Li et al. 2015). Environmental pollution caused by xenobiotics has become a big issue. Visual contaminants introduced into water systems by the textile and dye industries, in addition to creating color, are hazardous to aquatic and other living forms (Joshi et al. 2010). The textile industry is also one of the most water-consuming sectors, producing effluent with varied properties and a complicated nature (Spagni et al. 2012). Biological treatment of wastewater using bacteria has recently emerged as an important research area (Drogui et al. 2005; Cheung and Gu 2007). Microbes are nature's original recyclers, turning hazardous chemical substances into simpler, non-toxic products like carbon dioxide and water. The existence of a vast number of different bacteria, fungus, and other microorganisms in nature broadens the range of chemical pollutants that can be destroyed and the extent to which polluted places can be decontaminated by native microbes. Several studies have been conducted on dyestuff decolorization utilizing pure bacterial strains or a combination of selected strains (Oturkar et al., 2013).

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The biodegradation of xenobiotic compounds by microbial communities, which transfer substrates and products between each other and cooperate metabolically and also shows intra-species and inter-species horizontal transfer of DNA, has been known for a long time. They may be develop due to the exposure of recalcitrant chemicals over a long period of time, like in the case of direct draining of textile effluent into the Chalawa in Kumbotso and at other places of textile hub (Abraham et al. 2002; Arunprasad and Bhaskara Rao 2010; Faryal and Hameed 2005; Carliell et al. 1995; Wilkins 2002). Diverse industrial activities lead to heavy pollution of soils and surface waters by contributing heavy metals such as Chromate, which can be alleviated through bioremediation by resistant microorganisms (Fernandez et al. 2013). Table 5 shows the microbial diversity in wastewaters and effluents from different sources in different countries. Some microbes like free-living amoeba (Acanthamoeba, Echinamoeba, Korotnevella, etc.) were reported to be present in textile industrial wastewater which feed on bacteria (Ramirez et al. 2014). While in another study, various molecular and statistical methods were employed to obtain different microbial communities (*Acidobacterium, Actinomycetes, Proteobacteria*, etc.) from different domestic and industrial wastewaters (Boon et al. 2002). Gajera et al. (2015) have isolated fungi from effluent contaminated plant rhizosphere near textile dyeing industry and reported the decolorization and biodegradation of textile effluent by the novel fungi Hypocrea koningii. Our understanding and knowledge about microbial potential and exploitation of their metabolic processes must be channeled in proper application prospective way to mitigate the problems associated with industrial effluents and their pollution load. It has been suggested that the increasing amount of information available about the strains, compounds, enzymes and reactions implicated in microbial biodegradation of toxic pollutants provides us with the building blocks for formulating a 'biodegradation network' (Pazos et al. 2003).

Native Bacteria and Fungi, isolated from effluent sites, i.e., *Aeromonas sp., Pseudomonas sp., Flavobacterium sp., Rhodococcus sp.,* and fungal strains *Myrothecium sp. Phanerochaete chrysosporium* may have potential to absorb and degrade the dye component from textile effluent (Hu et al. 1992; Mou et al. 1991; Heiss et al. 1992; Glenn and Gold 1983). Pure bacterial strains, such as *Pseudomonas luteola, Aeromonas hydrophila, Bacillus subtilis, Pseudomonas sp.* and *Proteus mirabilis* decolorizes dye under anoxic conditions while in some cases they need additional carbon sources to decolorize as they are unable to utilize the dyes due to their toxicity (Chang et al. 2001). Apart from bacteria and fungi, a variety of free living amoeba is also reported to be present depending on the characteristics, i.e., content of colorants, surfactants of effluents of dyeing plants. They feed on bacteria and become the link between decomposing organisms and other higher organisms in the trophic level (Ramirez et al. 2014). The fate of dye stuff was investigated in biologically based primary treatment to understand the mechanism of biological potential in activated sludge and it was found that partial color removal was achieved by adsorption of the dyes to the sludge (even though they were water soluble). Also, subsequent removal by flocculation and the possibility of better results by adaptation of microbes in textile effluent medium (where the carbon source is only in the form of effluent) has been observed (Pagga and Brown 1986). Dye toxicity may restrict the microbial diversity of activated sludge and reduce the extent of color removal in treatment process (Brown et al. 1981).

A recent investigation in Chalawa has suggested the contamination of soil and sediment of river bed by different metals and dye stuff and also the adaptability of native microbial community to decolorize the color of effluent. The river bed soil and sediment (Prabha et al. 2013, 2014, Kumar et al. 2009, 2010). These changes can be attributed to high content of metal ions in various dyes (Arunprasad and Bhaskara Rao 2010). Thus, the strains show adaptability to severe conditions of the effluent and their survival in the highly contaminated water. The ability of the microbes to decolorize textile dyes has also been attributed to their adaptability to degrade the xenobiotic compounds by their biological activity and chemical structure of the dyes. The individual strains may attack the dye molecule at different positions or may use degradation products produced by other strains for further degradation (Coughlin et al. 1997). The addition of effluent initiates a series of physico-chemical changes in the water body and sediment, where all the pollutants get settled over time. It increases the chemical load in the system which in due course of time leads to the adaptation of microbes in the harsh conditions depending upon the type of chemicals present. The difference in microbial composition as well as its density in polluted and non-polluted water is due to chemical laden effluent and it is very clearly interpreted.

The objective of this paper is to study the changes in the pollution load flux in Chalawa River with respect to microbial population by comparing polluted and non-polluted sites and also to compare microbial population of treated and non-treated effluent in both the treatment systems.

Study area

This research was carried out in river Challawa that originates from the Challawa Goje dam in Challawa village within Kumbotso local government area of Kano state, Nigeria. It lies between 11^0 10 N to 11^0 22^0 N latitude and 77^0 21^0 E to 77^0 50'E longitude. Kano state occupies a central position in the northern Nigeria (Akan et al., 2007). The land use categories are classified as Built up, Agriculture, Water bodies and Waste land. Soil types in Chalawa can be divided into Fine, Fine loamy, Loamy skeletal, and Clayey loamy. Textile and Dyeing industrial units are the primary source of livelihood for the local as well as migrated skilled and unskilled workers. Industrially, it is one of the most developed cities in the region. It has three major industrial estates namely Bompai, Challawa and Sharada industrial estates. Each of them accommodates many wet industries. Tanneries and textile processing are some of the dominant industries.

Fig. 1 Map illustrating Chalawa industrial location and sampling site of Kumbotso, Kano State Nigeria

Materials and methods

Effluent water samples were collected from a highly polluted Chalawa Industrial site sewage and a far upstream non-industrialized stretch of Tamburawa water treatment plant, were analyzed. Secondly, I have done the comparative study of BT and AT effluents in the two treatment systems, i.e., conventional as well as MBR-based CETP, with respect to microbial population. For microbial diversity analysis, samples of effluent and surface water were collected in dry, sterile polypropylene bottles, which were kept in ice during transportation. Samples were stored in refrigerator (4 ^{0}C) for further laboratory process.

Isolation of bacteria and fungi by serial dilution and plate count Techniques

1 % NaCl concentration stock solution was prepared, and then serial dilution blanks were setup in test tubes and marked sequentially starting from 10^{-1} to 10^{-6} dilution and autoclave sterilized. 1 ml of water sample was transferred in 9 ml solution, i.e. 10^{-1} dilution. 1 ml from 10^{-1} test tube was then transferred to 9 ml of the 10^{-2} labeled test tube i.e. 10^{-2} dilution, using a fresh sterile pipette; and this was repeated for each succeeding step till 10^{-6} . Nutrient agar (NA) Agar media was used for the isolation of bacterial strains and for the isolation of fungal strains potato dextrose agar (PDA) media was used. From 10^{-3}, 10^{-4}, and 10^{-5} dilution tubes, 0.1 ml of dilution was then spread on sterilized petriplates in triplicates using the standard spread plate technique, for both bacterial and fungal strain isolation (Figs. 2). The NA agar plates were then incubated at 37 ^{0}C for 24 h and the PDA plates were incubated at 28 ^{0}C for 72 h. After successful growth of microorganisms, characteristics of each distinct colony, e.g., shapes, color, transparency, etc. were determined. The number of bacterial and fungal colonies in the water samples was counted and the density was expressed as Colony Forming Units (CFU).

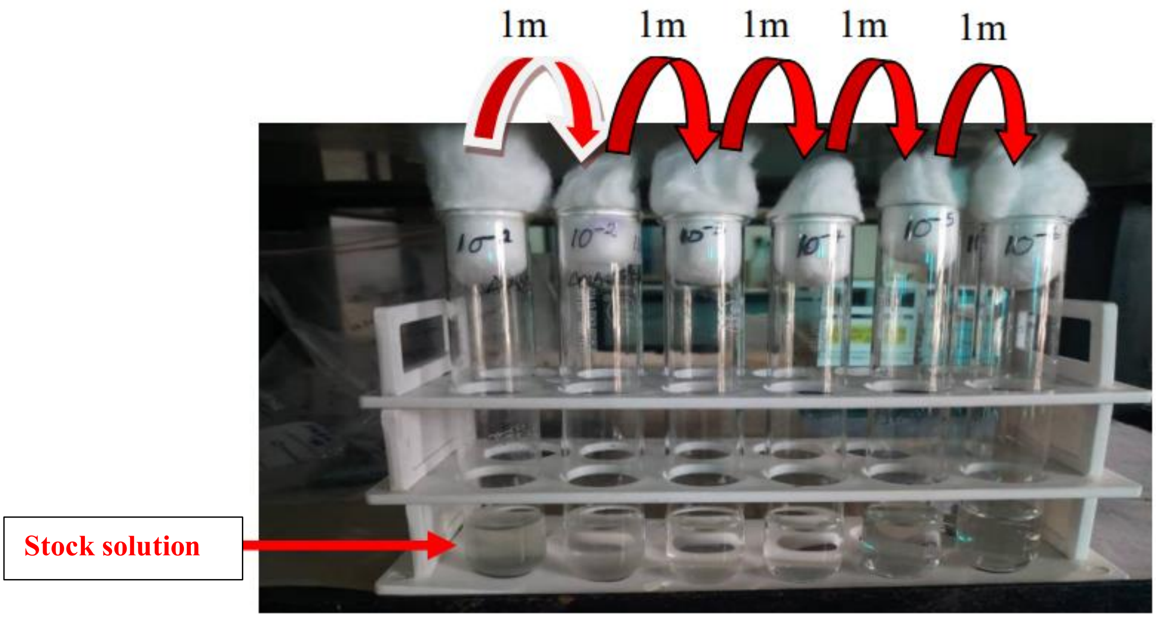

Fig. 2 Stepwise process of serially diluted sample at different dilution factor

Physico-chemical characterization of bacterial strain

The morphological analysis of the acquired colonies based on their form, size, color, opacity, texture, elevation, margin, nature under the microscope, and gram staining was used to assess the bacterial diversity of water and effluent samples. Furthermore, biochemical test was conducted, including the MRVP test, the catalase test, and the starch hydrolysis test (Seeley and Van Demark 1972). Following their appearance on the PDA plates, the fungal colonies were separated, purified, and described according to their morphological characteristics, such as the mycelia diameter, color, and texture.

Gram Staining of Bacteria and fungi

The technique was recognized by Hans Christian Joachim Gram, and involves a number of steps for staining bacterial cells with dye. Following fixing of bacterial cell, staining was conducted using dye; Crystal Violet, Gram Iodine, Ethanol and Safranin.

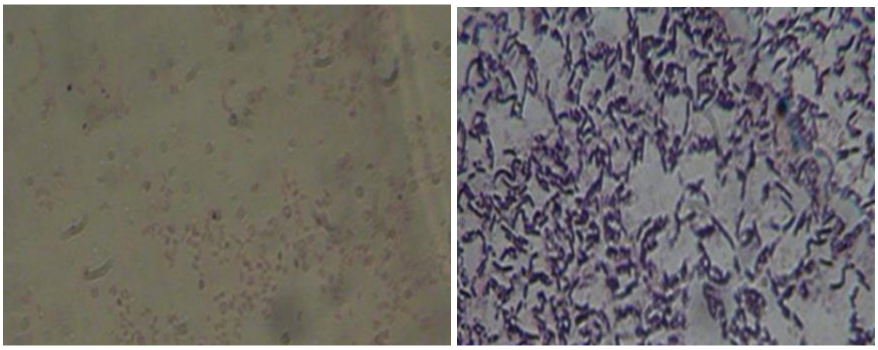

Fig. 3 Gram staining of *Pseudomonas sp., Achromobacter sp* at x100

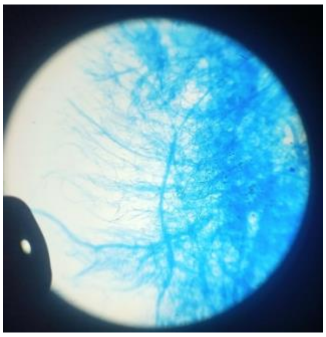

Fig. 4 Gram staining of fungus *Aspergillus fumigates*

Microscopic study of fungal strains

The fungal population was characterized to species level by referring standard mycological books and manuals on the basis of their morphological characters under microscopic observations using lacto phenol as a dye (Gilman 1959, 1998; Subramanian 1971, 1983; Ellis and Ellis 1985).

Results and discussion

The direct discharge of effluents into the Chalawa Dam may have implications over microbial population, both qualitatively and quantitatively. The Total Heterotrophic Bacteria (THB), e.g., *E. coli* and *Salmonella/Shigella* are indicators of water contaminants with domestic and pathogenic contamination (La Rosa et al. 2001), hence analyzed for the present study. The microbial diversity was expressed in Colony forming units per ml (CFU/ml) at the sampling sites and is shown in Table 1.

The total heterotrophic bacteria, *E. coli* and fungal count showed the contribution of industries in terms of high THB in surface water of industrial site as compared to the non-industrial site. Bacterial as well as fungal population was low in non-industrial site, while it got multiplied due to textile effluent discharge and domestic sewage contamination in Chalawa River. However, the population density of *Salmonella/Shigella* remained same in both upstream to Tamburawa water treatment plant as well as in the polluted industrial site, suggesting possible sewage contamination in the non-industrial site. This result suggests that microbial population in river water is enhanced by the industrial pollution load (Table 1). To compare the microbial load of BT and AT effluent, samples from both biologically based conventional, which are common in practice for waste water treatment in CETPs in Chalawa and MBR-based treatment system which has just started operating, were collected and analyzed. The microbial population of textile effluent estimated for BT and AT for conventional treatment system showed that bacterial population density in AT effluent is less (2.53 9 106 CFU/ml) as compared to BT effluent (6.11 9 107 CFU/ml). Similar trend has been observed for fungal population density, i.e., 1.5 9 103 CFU/ml in AT and 1.8 9 103 CFU/ml in BT effluent (Table 2).

This result suggests that the microbial population in AT effluent decreased compared to that of BT which may be because of the removal of nutrient constituent by degradation of effluent in the conventional treatment system. The microbial load of effluents after conventional treatment is very high, as microbial sludge settle through secondary clarifier under gravity, while in case of MBR-based treatment systems, membrane filter is used to separate sludge from the water. Ideally effluent after MBR-based treatment should be free from microbes and organic loads (Cicek et al. 1998). The output of primary treatment is taken for more advanced treatments like RO and microbial growth may cause fouling of membrane filter hampering the process. Thus, MBR-based treatment promises a suitable option for effluent treatment to achieve the norm of zero discharge in general. The microbial stock in BT effluent was higher than microbial stock of river water at Chalawa site because of the dilution effect of river to effluent or treated/semi-treated effluent. The bacterial count of AT effluent decreased a lot (from 3.91 9 107 CFU/ml to 1.2 9 102 CFU/ml) due to the removal of bacterial sludge in MBR system.

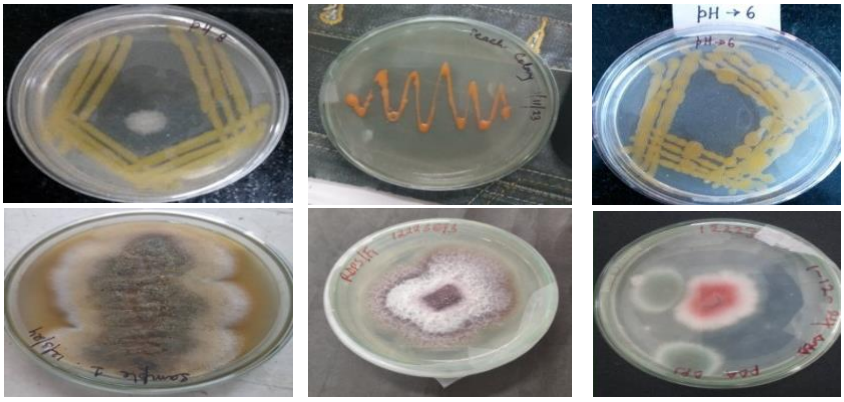

Fig. 5. Bacterial colonies obtained in a 10^{-3}, b 10^{-4}, c 10^{-5} dilution plates containing NA agar, d E.coli cells grown in Eosin-Methylene Blue agar, e and f fungal colonies obtained in PDA

Table 1 Microbial density at polluted and non-polluted sites of Chalawa Industrial area

Sample location	Bacteria CFU/ml			Fungi CFU
	THB	***E.Coli***	***Salmonella***	
Chalawa river	0.67×10^5	2.0×10^5	1.0×10^3	1.3 x
Tamburawa water	7.33×10^6	2.5×10^5	1.09×10^3	

Total heterotrophic bacteria (THB)

Table 2 Microbial density of textile wastewater in different Tamburawa water treatment plant

Treatment system	Bacteria CFU/ml		Fungi CFU/ml	
	BT effluent	**ATE effluent**	**BT effluent**	**AT effluent**
Conventional Biological	6.11×10^7	2.53×10^6	1.8×10^3	1.5×10^3
MBR	3.91×10^7	1.2×10^2	1.6×10^3	1.0×10^4

AT& BT represents after and before treatment effluent samples, respectively

To compare the microbial load of BT and AT effluent, samples from both biologically based conventional, which are common in practice for waste water treatment in CETPs in Chalawa and Tamburawa-based treatment system, were collected and analyzed. The microbial population of textile effluent estimated for

BT and AT for conventional treatment system showed that bacterial population density in AT effluent is less (2.53 x 10^6 CFU/ml) as compared to BT effluent (6.11 x 10^7 CFU/ml). Similar trend has been observed for fungal population density, i.e., 1.5 x 10^3 CFU/ml in AT and 1.8 x 10^3 CFU/ml in BT effluent (Table 2). This result suggests that the microbial population in AT effluent decreased compared to that of BT which may be because of the removal of nutrient constituent by degradation of effluent in the conventional treatment system.

Both the water samples show abundance of bacterial species like *E. coli, Bacilli sp., Salmonella Shigella sp*., etc. The high density of the bacterial population like *E. coli* and *Salmonella Shigella sp.* indicates pathogenic contamination along with industrial effluent drainage (USEPA 2000). Considerable amounts of toxic and complex dyes are discharged directly into the Chalawa River as effluent and also into wastewater treatment plants by industrial units, thus imposing a selective pressure on the microbial flora residing in wastewater habitats. Decolorization generally occurs by the adsorption of dyestuffs on bacteria, rather than oxidation in aerobic systems. Some bacteria can biodegrade dyestuffs by azo reductase activity (Chung and Stevens 1993). The effluent laden water of Chalawa as well as Tamburawa water treatment plant have bacteria like *Pseudomonas sp.* and *Achromobacter sp.*

Table 5 Microbial community in wastewater from different sources

Country/ location	Source	Microbial community	References
Famaillá, Tucumán, Argentina	Textile-dye effluent drainage	*Cyberlindnera jadinii*, *Wickerhamomyces anomalus*, etc.	(Fernández et al. 2013)
Mexico	Wastewater treatment plant	*Acanthamoeba*, *Echinamoeba*, *Korotnevella*, *Mayorella*, *Vermamoeba*, etc.	(Ramirez et al. 2014)
Flanders, Belgium	Domestic wastewater and wastewater from textile industry	Acidobacterium, Actinomycetes, Type I methanotrophs, Type II methanotrophs, α-Proteobacteria, etc.	(Boon et al. 2002)
China	Dyeing wastewater from moving bed biofilm reactor (MBBR)	*Caldilinea aerophila*, *Oscillibacter valericigenes*, *Caldilinea tarbellica*, *Bacillus* sp., *Nitrosomonas eutropha*, *Acidothermus cellulolyticus*, *Geobacillus thermoglucosidasius*, etc.	(Li et al. 2015)
China	Sea mud of industrial harbor	*Brevundimonas* sp., *Nitrospira* sp., *Bacillus aeolius*, *Thermomonas brevis*, *Brevibacterium* sp., etc.	(Tan et al. 2013)
China	Dye-contaminated water	*Klebsiella sp.*, *Escherichia sp.*, *Bacillus* sp. and *Clostridium* sp.	(Cui et al. 2012)
China	X-3B dye wastewater	*Bacillus sp.*, *Sedimentibacter sp.*, *Pseudomonas sp.*, and *Clostridiales*, *Streptomyces.*	(Tan et al. 2009)
India	Effluent contaminated plant rhizosphere near textile dyeing industrial area	*Trichoderma viride*, *Trichoderma koningii*, *Hypocrea koningii*, *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium oxysporum*	(Gajera et al. 2015)

CONCLUSION

The microbial density and diversity were observed to be higher in the river water at site near the industrial hub as compared to the upstream site. Similarly, the microbial populations were found to be higher in BT effluent than AT effluent for both the treatment systems. The river site near industrial hub and the upstream site had similar biological diversity. The bacteria (*Pseudomonas sp.,* and *Achromobacter* sp) and the fungus (*Aspergillus fumigates*) found in river water were reported to have decolorization potential of dye effluent. Thus, the findings may help the fund to beneficially use these strains and other related microbes in decolorizing and thereby detoxifying treatment of various dye containing effluents prior to discharge or reuse. In this study only chemical and microbial indicators were taken into account. Dye effluents are not only toxic to the aquatic biota but also carcinogenic for human beings and once they get into the water system, possess potential threat to life.

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Data Availability Statement: The data presented in this research are available on request from the author.

Conflicts of Interest: The author have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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