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A Study on Biodegradation of AZO Dyes by Microorganisms

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Abstract: *Dyeing is the application of dyes or pigments on textile materials such as fibres, yarns and fabric with the objective of achieving color with desired fastness. Dyes are obtained either naturally or from synthetic sources. Disposal of dyes into aquatic environment causes serious damage and may be toxic to aquatic organisms. They can cause immune suppression, reproductive failure or acute poisoning. It also upsets the biological activity in aquatic life and also the photosynthesis process of aquatic plants and algae. Physical methods like filtration, flocculation has been used widely but it is expensive and discharges chemicals into the stream which is again toxic. Use of microorganisms for dye degradation has been in practice which gives effective results in a cost effective and efficient way. The study explains the use of bacteria and yeast in dye degradation in a less amount of time yet an effective one and a comparative study of various physical and biological parameters including pH, DO, BOD, COD and TDS and use of nutritional sources.*

Key Words: *Bacterial isolates, BOD, COD, Dye decolourisation, Effluent water, TDS.*

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I. Introduction

Water is essential for survival and existence of life on planet earth. Over the last few decades increasing urbanization and industrialization has caused different environmental pollution. Color is the most visible indicator that can be easily recognized in waste water and it should be treated properly before discharging, because the effluents from the textile industry contains toxic chemicals such as azo dyes and reactive dyes which adversely affect the natural resources. Increased demands for textile products leads to increase in generation of wastewater, which makes the textile industry a main source of water pollution problems worldwide (Asia *et al*., 2006) Dyes can be obtained from natural sources such as microorganisms (Rajendran and Thamaraiselvi, 2014) vegetative matter, mineral or are manufactured in the factory from petrochemical feedstock. Direct dyes are cheap and easy to apply, but of poor fastness quality and so to improve the quality, synthetic dyes were discovered. Today, synthetic dyes are being extensively used in both textile and dyeing industries, because of their ease and cost effectiveness. However, among various industries, textile industries are considered one of the major consumers of dyes in the market (Namdhari *et al*., 2012). These industrial wastes play a significant role in water pollution. Discharge of these effluents in aquatic systems not only causes formation of toxic aromatic amines under anaerobic conditions but also have an adverse effect on terms of chemical oxygen demand. Further, many of these dyes are toxic, mutagenic and carcinogenic besides interfering with the transmission of sunlight and reducing photosynthetic activity (Campos *et al*., 2001, Millan *et al.,* 2001) in addition to their visual effect. Degradation of dyes is difficult due to their complex structure and nature. Various physical, chemical and biological methods like adsorption, sedimentation, flocculation, neutralization, electrolysis, ion exchange etc are employed to remove color. Use of these methods involves huge costs besides accumulation of large quantities of sludge along with and removal of these dyes from textile mill effluents is still a major challenge. Among the economic and environmental friendly techniques used for removal of colour or dye from dye waste water effluents, bioremediation technology has gained considerable attention in many countries. Bioremediation using microorganisms like bacteria, fungi, algae and yeast are most used in the treatment of dye effluents. Among these, bacteria are most commonly used for bioremediation process (Rai *et al*., 2005). Some bacteria can also degrade dye stuffs by azo reductase activity. However, the effluent at the end, dye stuff can become toxic. These problems limit the large scale application of bacterial decolourisation. Bacterial strains including *Bacillus sp., Pseudomonas sp.,* (Ewa Zablocka *et al.,* 2014), *Klebsiella sp., Escherichia coli., Staphylococcus sp.*,(Kamran Ali Khan and Seweta Srivastava, 2014) and Baker's Yeast (M.S. Mahmoud, 2016) were used for the treatment of textile effluent water. Optimization of

various parameters including p^H, nutritional sources, aeration conditions were recorded and compared by using various bacterial strains.

II. Materials And Methods

2.1 COLLECTION OF TEXTILE SAMPLE AND SOIL SAMPLE

The four industrial effluent samples were collected from Tirupur District and bacterial isolates were collected from soil. Baker's Yeast was collected from the local market, Coimbatore. Organisms were inoculated on Selective media. The colonies were observed and maintained by sub-culturing at regular intervals for further use.

2.2 CHARACTERIZATION OF UNTREATED AND TREATED EFFLUENT SAMPLES

The raw effluent was characterized by measuring the values of different physico-chemical parameters. All the physico-chemical analyses were done immediately after the effluent was collected from the industrial site (Rajendran, *et al..*, 2016). The two principle methods of measuring TDS are gravimetric analysis and conductivity. Gravimetric methods are the most accurate and involve evaporating the liquid solvent and measuring the mass of residues left.

2.3 PHYSICAL PARAMETERS OF EFFLUENT SAMPLES

The dye effluent sample was used for various Physico-Chemical analysis viz Color, Odour, p^H, Spectrophotometric scanning, Biological oxygen demand, Chemical oxygen demand and Total Dissolved Solids.

2.5 DISSOLVED OXYGEN OF THE SAMPLES

Dissolved oxygen present in the dye effluent sample was measured by Wrinkler's method. According to the method the quality of iodine liberated is directly proportional to the concentration of dissolved oxygen, which can be measured by using strong reducing agent like sodium thiosulfate.

2.6 BIOLOGICAL OXYGEN DEMAND OF THE SAMPLES

Biological oxygen demand was done to measure the amount of oxygen consumed in the biological processes that break down organic matter in effluent water, which is the measure of the organic pollutant load. Less the BOD value is better for aquatic flora and fauna. High BOD levels results in anoxic conditions, with the result of growth of anaerobic microorganisms that produces noxious gases causing death of aquatic organisms. Dissolved oxygen present in the dye effluent samples was measured by Five day Biochemical Oxygen Demand by Delzer and McKenzie (2003). According to the method the quality of iodine liberated is directly proportional to the concentration of dissolved oxygen, which can be measured by using strong reducing agent like Sodium thiosulphate.

2.7 CHEMICAL OXYGEN DEMAND OF THE SAMPLES

Chemical oxygen demand was done to measure the amount of oxygen that can be consumed by reactions in a measured solution. The estimation of COD is of great importance for waters having unfavorable conditions for the growth of microorganisms, such as presence of toxic chemicals. COD is always higher than BOD, by approximately two to three times of BOD. COD test was done for all the four samples. COD was done for all the four samples (S1, S2, S3, and S4).

2.8 BIOLOGICAL PARAMETER OF THE SAMPLES

The collected dye effluent samples were serially diluted and plated on nutrient agar plates using spread plate technique. The plates were incubated at 37˚C for 24 hours. Rose Bengal plates were prepared for fungal colonies.

2.9 OPTIMIZATION STUDY FOR DYE DEGRADATION

The optimization study was done for all the four samples to find the best decolourization and degradation occurring at different sugar concentration levels, in different pH and in different aeration conditions using different organisms.

2.10 EFFECT OF AERATION ON SAMPLES

Samples were placed in both incubator and in incubator with aeration at 120 rpm (Dipankar Chandra Roy *et al.,* 2018) at 37˚C for 7 days with 1ml of organism and different concentration of sugars. The sample degradation were checked and compared by decolourisation and spectrophotometric analysis.

2.11 EFFECT OF pH ON DEGRADATION

Dye effluent 1 (Sample 1) was inoculated with 1ml of different bacterial cultures along with different sugars and at different pH such as 6.0, 7.0, 8.0, 9.0, 10.0, 11.0 and 12.0. The sample was then incubated at 37˚C for 7 days and the results were checked for alternative days using UV spectrophotometer at 500nm.

2.12 EFFECT OF NUTRIENTS ON DEGRADATION

Different carbon sources such as Glucose, Fructose, Sucrose and Lactose were taken at different concentrations from 0.5g, 1.0g, 1.5g, 2.0g (Sylvine Lalnunhlimi, *et al*.., 2016) and added to 25 ml of the different sample effluents as a supplement. The cultures of 1ml were inoculated into the different samples containing carbon source and incubated at 37˚C for 7 days. Decolorization was checked visually and percentage was also calculated on 1st, 3rd, 5th and 7th day at 560nm and 700nm.

III. RESULTS AND DISCUSSION

3.1 BIOLOGICAL PARAMETER OF THE SAMPLES

Biological parameter of the samples S1, S2, S3 and S4 were done and results are tabulated. Nutrition agar plates were prepared for Bacteria and Rose Bengal plates for Fungi.

TABLE 1: BIOLOGICAL PARAMETER OF THE SAMPLES

Sample number	Medium	Incubation days	Incubation temperature	Growth
S1	Nutrient medium Rose Bengal medium	5 5	37˚C Room temperature	No growth No growth
S2	Nutrient medium Rose Bengal medium	5 5	37˚C Room temperature	No growth No growth
S3	Nutrient medium Rose Bengal medium	5 5	37˚C Room temperature	No growth No growth
S4	Nutrient medium Rose Bengal medium	5 5	37˚C Room temperature	No growth No growth

Growth was not observed in the plates even after 5 days of incubation with the nutritional source provided which indicates, that the effluent water has huge quantities of chemical load in it which makes it unfit for any usage. Daniel and Richard, 1973, reported that the test had effect on dye due to the growth of microorganisms. The results indicated that readily available dyes might have potential application for selective isolation of specific bacterial groups than from the effluent dye waste waters.

3.2 DETERMINATION OF PHYSICAL PARAMETERS OF THE SAMPLES

Color is very important factor for the aquatic life for making food from sun rays. The photosynthesis activity reduced due to dark coloration which affects parameters like temperature, DO, BOD, COD, etc.

TABLE 2 PHYSICAL PARAMETERS OF THE SAMPLES

Sample number	Colour		pH		Odour	
	Before degradation	After degradation	Before degradation	After degradation	Before degradation	After degradation
S1	Greenish Blue	Greenish Blue	6.0	6.0	Odourless	Odourless
S2	Red	Pale Yellow	12.0	8.2	Unpleasant	Unpleasant
S3	Dark Orange	Pale Yellow	12.0	7.6	Unpleasant	Odourless
S4	Dark Red	Pale Yellow	14.0	7.0	Odourless	Odourless

The samples were decolourised to pale yellow from red, orange and dark red (Plate1, 2, 3). The p^H of the samples was brought to neutral levels from alkaline levels (table 2). The odour of the samples was converted to odourless from unpleasant smell. These changes prove that microorganisms can degrade the effluent water and reduce the $p^{H.}$ Similar results were recorded by Arumugam and Sivagami, 2016.

3.3 TOTAL DISSOLVED SOLIDS OF THE DEGRADED SAMPLES

The total dissolved solids present in water are one of the leading causes of turbidity and sediments in usable water. When left untreated total dissolved solids can be the cause of various diseases. When TDS levels exceed 1000mg/L and generally considered to be unfit for any kind of usage and from the below results it can be clearly observed that microorganisms were able to reduce the TDS levels by 60% - 70%.

TABLE 3: TOTAL DISSOLVED SOLIDS OF THE SAMPLES BEFORE AND AFTER DEGRDATION

Sample numbers	Organism used	Before treatment(mg/L)	After treatment(mg/L)
S1	(i)*Escherichia coli* (B1)	1082	1082
	(ii) *Klebsiella sp.*, (B2)	1082	1082
	(iii)*Pseudomonas sp.,* (B3)	1082	1082
S2	(i)*Escherichia coli* (B1)	1024	310
	(ii) *Klebsiella sp.*, (B2)	1024	344
	(iii)*Pseudomonas sp.,* (B3)	1024	334
S3	(i)*Escherichia coli* (B1)	736	307
	(ii) *Klebsiella sp.*, (B2)	736	398
	(iii)*Pseudomonas sp.,* (B3)	736	425
S4	(i)*Bacillus sp.*, (B4)	1218	566
	(ii)*Staphylococcus sp.*, (B5)	1218	370
	(iii)*Yeast* (Y1)	1218	467

The TDS levels of the samples were reduced incredibly after being inoculated with microorganisms. The results represents that the textile effluent water can be degraded by microorganisms successfully and more effectively within a short period of time. Hanie *et al.*, (2017) performed experiments to prove microorganisms can reduce TDS levels in effluent waters.

3.4 BOD AND COD OF THE EFFLUENT SAMPLES

The BOD of normal water is 1mg/L and levels less than 8mg/l are considered to be moderately polluted and levels more 8mg/L are considered to be highly polluted. The COD of normal water is less than 250mg/L. COD of sewage water normally ranges from 600-900mg/L. and it must be treated to at least 75-100mg/L before discharge to minimize pollution potential. Using microorganisms to degrade the effluent water has shown noticeable decrease in BOD and COD levels of the treated water shown in Table 4.

TABLE 4 BOD AND COD OF SAMPLES BEFORE AND AFTER DEGRADATION

Sample number	Microorganism	Initial BOD (mg/L)	Final BOD (mg/L)	Initial COD(mg/L)	Final COD(mg/L)
S1	(i)*Escherichia coli* (B1)	20	20	1050	1050
	(ii) *Klebsiella sp.*, (B2)	20	20	1050	1050
	(iii)*Pseudomonas sp.,* (B3)	20	20	1050	1050
S2	(i)*Escherichia coli* (B1)	23	8.4	950	300
	(ii) *Klebsiella sp.*, (B2)	23	9.2	950	320
	(iii)*Pseudomonas sp.,* (B3)	23	9	950	403
S3	(i)*Escherichia coli* (B1)	22	8	835	382
	(ii) *Klebsiella sp.*, (B2)	22	10	835	406
	(iii)*Pseudomonas sp.,* (B3)	22	7	835	402
S4	(i)*Bacillus sp.*, (B4)	18	8	1350	709
	(ii)*Staphylococcus sp.*, (B5)	18	7	1350	386
	(iii)*Yeast* (Y1)	18	9	1350	420

BOD and COD of the samples were checked both before and after degradation. The results prove that both BOD and COD levels of the textile effluent waters have been greatly reduced after being treated with microorganisms. Sample 2 has been degraded by *E.coli* (B1), Sample 3 has been degraded best by *Pseudomonas sp.*, (B3) and Sample 4 has been degraded by *Staphylococcus sp.*, (B5). BOD and COD values have been reduced by nearly 60% of its original values. Similar results were observed by Arumugam and Sivagami (2016). The maximum reduction was recorded in the medium containing the combination of bacteria and fungi where the BOD level decreased from 350mg/L to 108mg/L showing 65.1% reduction.

3.5 EFFECT OF DIFFERENT CARBON SOURCES ON DEGRADATION

Initially the sample was inoculated with 100ml of nutrient broth to check for decolourisation. The dye was decolourised in three to four days. For 1ml of the sample 99ml of nutrient media was required for degradation to occur which is an expensive process when gallons of effluent water has to be degraded. So an alternative method of adding only carbon sources was carried out which resulted in good degradation in two to three days for different dyes using different carbon sources and different microorganisms (Table 5).

TABLE 5 EFFECT OF NUTRITIONAL SOURCE ON DYE DEGRADATION

Sample number	Organism	Carbon source	Amount of carbon source (g/25ml of the dye)	pH	Time taken for degradation (days)	Temperature
S2	(i)*Escherichia coli* (B1) (ii) *Klebsiella sp.*, (B2) (iii)*Pseudomonas sp.*, (B3)	Glucose Glucose Fructose	2.0 2.0 1.0	7.0 7.0 7.0	3 5 5	37˚C 37˚C 37˚C
S3	(i)*Escherichia coli* (B1) (ii) *Klebsiella sp.*, (B2) (iii)*Pseudomonas sp.*, (B3)	Sucrose Fructose Sucrose	0.5 1.0 0.5	7.0 7.0 7.0	3 5 3	37˚C 37˚C 37˚C
S4	(i)*Bacillus sp.*, (B4) (ii)*Staphylococcus sp.*, (B5) (iii)*Yeast* (Y1)	Lactose Glucose Glucose	1.0 0.5 1.0	7.0 7.0 7.0	3 3 7	37˚C 37˚C 37˚C

Sample 2 and Sample 3 showed best degradation on 3rd day of incubation at 37˚C and it was inoculated with *E.coli* (B1) with the supplementation of 2% Glucose and 0.5% Sucrose as Carbon source which is shown in Figures 1 and 2 and Plates 1 and 2 respectively. Sample 4 showed best degradation on 3rd day of incubation at 37˚C when it was inoculated with *Staphylococcus aureus* (B5)and supplemented with Glucose as carbon source (Plate 3). Maulin *et al.*,, 2013 reported that the carbon sources supplementation to the microorganisms can degrade dyes upto 95% .

FIGURE 1 EFFECT OF NUTRITION AND INCUBATION PERIOD ON SAMPLE 2

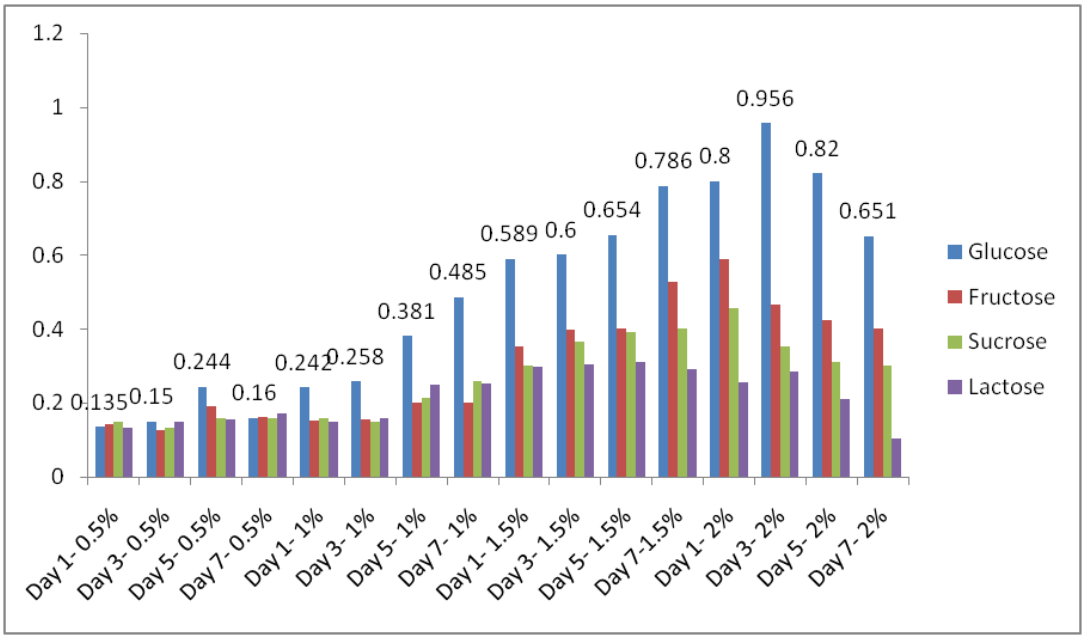


PLATE 1 SAMPLE – 2 Effect of Glucose on *Escherichia coli*

DAY 1 **DAY 4**

FIGURE 2 EFFECT OF NUTRITION AND INCUBATION PERIOD OF SAMPLE 3

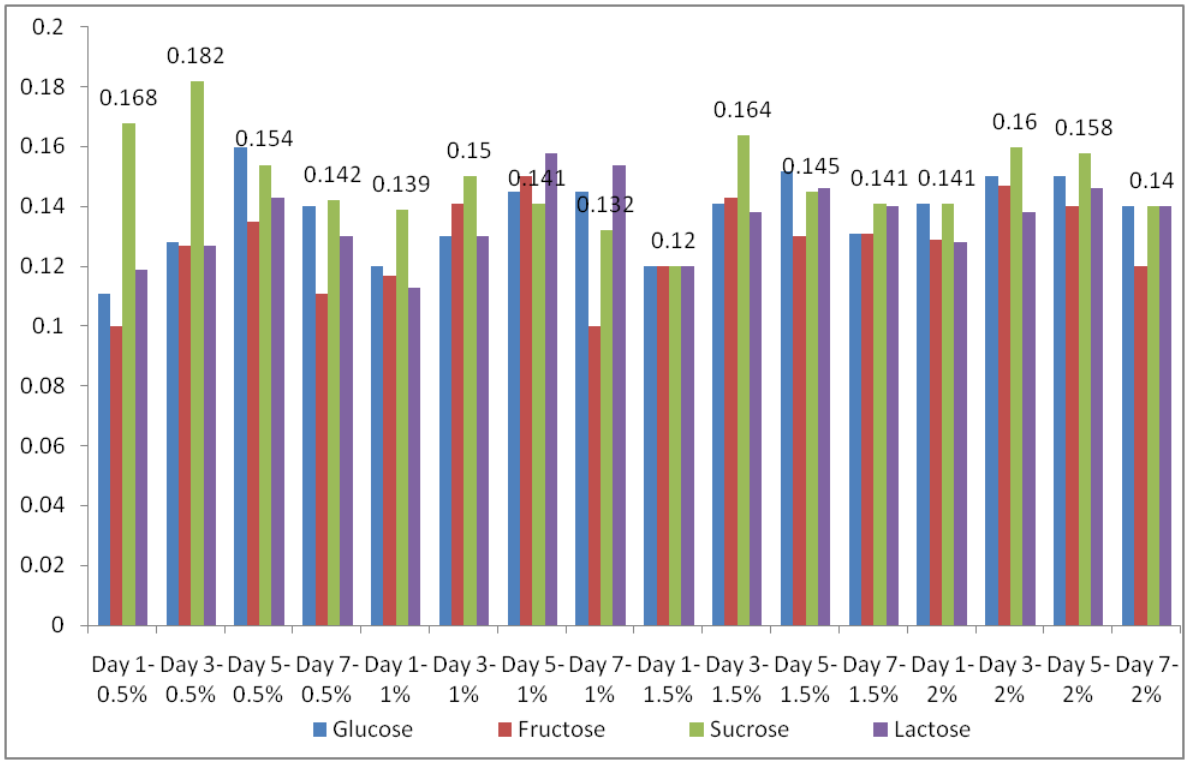
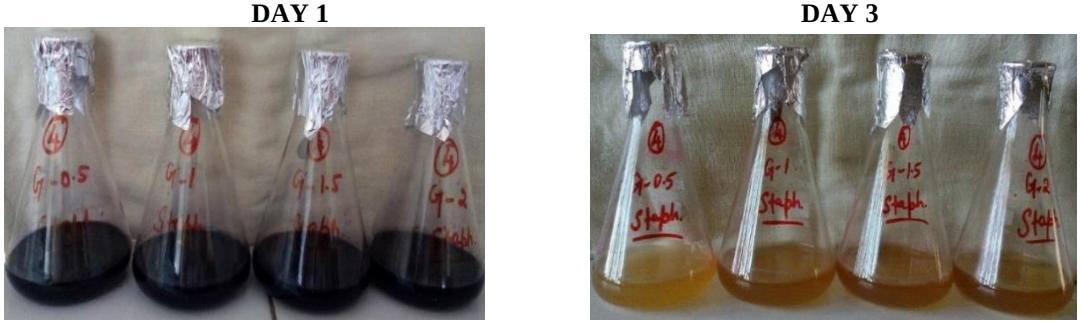


PLATE 2 Sample – 3 Effect Of Sucrose on *Escherichia coli*

DAY 1 **DAY 3**

PLATE 3 Sample – 4 Effect of Glucose on *Staphylococcus sp.,*

DAY 1 **DAY 3**



Similar results were observed by using sugars as a sole carbon source by Sylvine and VeenagayathrI, 2015.

All the above tests were done in both static and aeration conditions. Sample 2 showed best result when incubated with aeration. The results represents that the sample placed in shaker incubation showed less degradation than the sample placed in the static incubation. Bacterial species *Bacillus sp., Escherichia coli., Pseudomonas sp., Klebsiella sp., Staphylococcus aureus* exhibited better decolourisation than the Yeast which may be attributed to the presence of oxygen which normally inhibits the azo bond reduction activity since aerobic respiration may dominate utilization of NADH; thus impeding the electron transfer from NADH to azo

bonds (Nazeeha Ayaz *et al.*, 2012). UV visible spectrophotometer and Bio spectrophotometer were used to take the OD values. The OD values ranged from 500nm – 700nm depending on the color range of the sample.

IV. Conclusion

The development in industrialization leads to the release of more effluent into streams causes health hazards to aquatic microorganisms, aquatic flora as well as humans and also pollutes the soil and in turn effects the environment. Treatment of the effluent found to be beneficiary to the environment. Fermentation of effluent water using microorganism has been used widely because of its effectiveness and Ecofriendly nature. Microorganisms were found to degrade more efficiently and quickly when it has been supplemented with nutritional sources. In the research article the use microorganisms for degradation of toxic compounds to be cost efficient and also helps in maintaining the Ecosystem in a safer zone.

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