ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 2, pp: (481-494), Month: April - June 2019, Available at: www.researchpublish.com

Assessment of biodegrading potential of *Arthrobacter* isolated and identified from sewage and soil samples of Ulhas river basin

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Abstract: **The key to unravel most of the challenges associated with biodegradation and bioremediation of polluting substances may lie with autochthonous (indigenous) microorganisms present in polluted environments. Such bacteria can be isolated from stressed environments like sewage.They can also be found in soil habitat. Arthrobacter is one organism which is a common occurant in these habitats.These are pleomorphic organisms, exhibit typical rod to coccus morphogenesis and can degrade a wide range of organic & inorganic compounds, dyes and several pollutants. Forty five samples of sewage and soil were collected from Ulhas river and its basin .Organisms were isolated on Arthrobacter medium with pyridine. Preliminary identification of these isolates was done by Gram staining and biochemical characteristics. Further confirmation was done by phase contrast microscopy and their morphogenesis also was studied subsequently.Qualitative biodegradative screening of various organic as well as inorganic pollutants, pesticide, dyes and lignocellulosic materials, by these isolates, was checked. Twelve potent isolates were also studied for quantitative analysis of one dye(methyl orange) ,one inorganic pollutant(Chromium) and one organic pollutant(Acetonitrile. The activities of these isolates were constantly compared with those of Standard strains of Arthrobacter. Eighty five isolates were obtained from above mentioned samples. Morphology of thirty isolates matched with that of Arthrobacter. Sixteen isolates showed strong positive results of biochemical and morphogenetic characteristics .Identification was confirmed with 16s rRNA technique. All sixteen isolates could grow in presence of at least twelve out of nineteen contaminants.used for biodegradative screening. On quantitative analysis the percentage degradation was found to be upto 90% for dye, 76% for inorganic pollutant and 80% for organic pollutant.Thus Arthrobacter could go a long way in environmental cleanup of pollutants.**

Keywords: **Biodegradative, qualitative, morphogenesis, dyes, inorganic.**

1. INTRODUCTION

Arthrobacter are coryneform bacteria characterized by pleomorphism. They have a complex life cycle marked by two distinct stages,coccoid morphology during their stationary growth phase and rods in exponential growth phase[1]. *They* are nutritionally versatile, using a variety of substrates in their oxidative metabolism including various organic and inorganic pollutants, herbicides and pesticides.Many species of *Arthrobacter* are chemoorganotrophs, obligate aerobes and hence exhibit a pure respiratory metabolism, never fermentative one[2]. Ubiquitous in soil , several species have also been found in caves, sediments ,sewage, saline and water deficient environments. Most of the species can grow at 25-30^{o}C which is the optimum growth temperature of *Arthrobacter*. Some psychrotropic strains develop cold shock and cold acclimation proteins, allowing them to sustain life at low temperatures[3].Some species of *Arthrobacter* have adapted oxygen independent growth strategies in order to survive periods of oxygen limitation. *Arthrobacter* are extremely competitive organisms with remarkable resistance to dessication and starvation.[2]*Arthrobacter* can degrade wide variety of substrates which can be organic and inorganic pollutants. Some of the organic pollutants are food-processing wastes

like fat and grease, detergents, chemical wastes such as dyes, industrial solvents, cosmetic products, petroleum hydrocarbons (gasoline ,diesel & jet fuel, oil & motor oil), fuel combustion products from storm water run-off, insecticides and pesticides[4,5].Metals such as chromium, mercury, calcium, cobalt, iron, manganese, copper, potassium, magnesium, zinc, sodium & nickel are some of the inorganic pollutants[6] .They can also degrade naturally existing pollutants such as hydrocarbons, lignocellulosic wastes and decaying organic matter[7].The major threat to our planet is. pollution of environment. Pollution of valuable water supplies is especially important. Massive amounts of contaminated wastewater is produced annually by electric utilities, oil refineries and chemical factories. The groundwater gets polluted as a result of excessive fertilizer application e.g. phosphates,,nitrates and, and through leaching of naturally occurring trace elements in the soil after irrigation in agricultural processes. Pollution of both water and soil poses a significant hazard to human health and can lead to ecotoxicity.[8]Sewage treatment plants and industrial wastewater treatment plants are usually required to protect water bodies from untreated wastewater. Water pollution can also be prevented by erosion control from construction sites and agricultural wastewater treatment for farms [9]. A number of methods are used by industries and civic bodies to treat these polluted waters. Several physical and chemical water treatment processes available are adsorption, biosorption, flocculation, membrane filtration and advanced oxidation, for removal of pollutants from the system. Biological methods include primary-secondary sludge treatments, anaerobic digestions, aerobic treatment, activated sludge processes of sludge.[10] However physicochemical methods are very costly, less efficient and need specialized reactors. Disposal of secondary sludge generated during treatment is an added problem. Related microbiological technologies for treatment are bioremediation bioventing, landfarming, composting and biostimulation. Bioremediation is a process used to treat contaminated water, soil and subsurface material, by altering environmental conditions to stimulate growth of microorganisms and degrade the target pollutants.It is less expensive and more sustainable than other remediation alternatives.[11,12]. *Arthrobacter* is one such organism which can aid in bioremediation as it can utilize wide variety of pollutants as substrates.Though bioremediation of metals such as mercury,lead and cadmium by *Arthrobacter*, has been reported ,it plays an important role in removal of toxic form of Chromium[6] Hexavalent chromium causes severe irritaions to humans.It seeps into ground water very easily.It is hundred times more toxic than trivalent chromium.Very few organisms can grow in the presence of hexavalent chromium. *Arthrobacter* can not only grow in the presence of hexavalent chromium but also reduce it to trivalent chromium its less toxic form [6]. Because of its unique lifecycle and ability to survive under extreme conditions of environment,it is extremely competent microbe. It can be explored to remediate subsurface pollution and environmental cleanup by bioremediation, a cost and treatment-effective,non-destructive technology[13]. These microorganisms are potential candidates for bioremediation due to their ability to metabolize a wide range of of substances i.e; nasty chemicals &organic compounds as well as their ubiquitous presence in soil.Many conventional microorganisms are unable to operate as efficiently as these under these adverse conditions.Thus *Arthrobacter* might promise to be a better organism for carrying out bioremediation of sewage and soil .

2. MATERIALS AND METHODS

Sampling and Isolation

Collection of Soil samples

Soil samples were collected in Ziplock bags from agricultural fields of Ambernath, Ulhasnagar, Badlapur and Thane cities of Maharashtra, India. These samples were refrigerated till further processing.

Collection of Sewage and Sludge Samples:

The sewage and sludge sample samples were collected in sterile borosilicate bottles from different sites of Waldhuni Nallah (Ulhasnagar) and also from various Lakes and Ponds of Thane district, Maharashtra.

Enrichment and isolation:

The above-mentioned samples (Soil, Sewage and Sludge) were enriched in the *Arthrobacter* medium containing Pyridine as a selective component[14] The enrichment broth containing samples, were incubated on shaker for 5-7 days at room temperature. After two enrichments, loopful of enriched broth was used for isolation of *Arthrobacter* which was carried out on the same medium supplemented with agar. Plates were then incubated at room temperature and colonies thus obtained were purified and maintained on *Arthrobacter* medium slants for further studies

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Identification of the Isolates

Biochemical and Morphological characteristics

Isolates were identified based upon their colony characters, gram staining and biochemical test, with the help of Bergey's manual of bacteriology. Biochemicals carried out.were sugar degradation(glucose), catalase, gelatinase, cellulase and oxidative-fermentative tests. [15]. Morphogenesis of the isolates was studied with Phase contrast microscope (PCM)wherein wet mount of isolates were prepared and checked for changes in morphology'

The observations were recorded in photographs clicked with Sony DSC-W690 (16.1 Megapixels). The *Arthrobacter* isolates were thus confirmed by presence of rod-to-coccus morphology. These isolates were segregated from all the isolates capable of growing in selective medium with pyridine as the sole source of carbon.

The isolates seggregated by PCM appeared to be matching with the rod to coccus morphology of *Arthrobacte*r .However they were cross- checked by PCM for morphogenesis. These observations were made at 0,2 and 24 hours.*Arthrobacter* culture shows morphogenesis within few hours.Thus final level segregation was done on the basis of these cumulative observations.[16,17]

Biodegradative Screening/ Potential.

Qualitative biodegradation studies were performed by Plate assay method. The pollutant/compound was incorporated in different concentrations in respective agarified medium with the compound to be degraded as sole source of carbon. Concentrations of the selected compounds are given in the table below [14]

The results were observed as growth/no growth of the selected isolates, in presence of respective pollutant/compound at 24- 48 hours of incubation at R.T.

Group of compounds	Names of compounds/ Pollutants	Concentration of pollutant/compound & respective medium
Organic compounds	Phenol,Toluene, Benzene , Ethyl acetate, Aniline, Nicotinic acid, Xylene	5mg/ml each in Mineral Salts Medium(MSM)
Nitrogen containing Organic compounds	Trimethylamine, Diphenylamine, Acetonitrile, Picoline(methyl pyridine)	5mg/ml each in MSM
Dyes	Methyl orange, Methyl green, Methyl blue, Methyl violet	Dye Decolorization Malt Extract Agar Medium Industrial Dye-conc.0.2mg/ml.
Lignocellulosic waste	Sawdust, Lignin, Carboxymethyl Cellulose Xylan	5mg/ml each in Macbeths medium 2mg/ml
Organochlorines	Pentachlorophenol	100 mcg/ml in MSM
Inorganic compounds:	Chromium as potassium dichromate & Mercury as Mercuric Chloride	100mcg/ml each in Yeast Extract Agar Medium

Quantitative degradative analysis

Isolates which could degrade maximum number of compounds in the above qualitative screening were taken for quantitative degradation studies. The compounds analysed were methyl orange, chromium and acetonitrile.

Degradation/decolourisation of Methyl orange

100ml of Mineral salts medium (MSM) with 0.1% Yeast Extract and 200mcg /ml of methyl orange was used. The isolates and the standard cultures of Arthrobacter were inoculated and the flasks were incubated at R.T for seven days. The decolorized broth was centrifuged at 10,000 rpm for 10 min. and the clear supernatant was used to determine the optical density at λmax of the selected dye. The %ge decolorization was calculated using formula % Decolorization = (OD at T_0 – OD at T_t / OD at T_0) X 100 .Where T_0 is OD at Initial '0' Hours and T_t is OD after Time 't' Hours.[18]

Degradation of **inorganic compound**-Chromium.

100ml of Mineral salts medium (MSM) with 100mcg/ml of potassium dichromate was used .The selected isolates and the standard cultures of *Arthrobacter* were inoculated and the flasks were incubated at R.T for 5 days. Aliquots were removed from medium at regular intervals and hexavalent chromium estimation was done by Diphenyl carbazide method In acid solution, diphenylcarbazide and hexavalent chromium form a soluble red- violet product that absorbs light at 540 nm. Standard straight line graph was plotted by using set of potassium dichromate standards ranging from 5-100mcg/ml. Concentration of hexavalent chromium was determined from standard graph .Hexavalent chromium concentration was calculated as follows: Cr^{+6} (mcg/L)=1,000 X mcg Cr^{+6} in sample/ mL of sample.[12,19]

Degradation of organic compound--Acetonitrile:100ml of Mineral salts medium (MSM) with 102.5mcg/ml acetonitrile was used in this experiment.The potent isolates and the standard cultures of *Arthrobacter* were inoculated and the flasks were incubated at R.T for 5 days. The microbial degradation of nitriles is a 2 step mechanism. The first step involves conversion of simple aliphatic nitriles to corresponding amide via nitrile hydratase, which is followed by conversion of amide to carboxylate with release of ammonia via amidase.[20]The amount of ammonia released is directly proportional to concentration of acetonitrile degraded.Thus ammonia release, growth, and change in pH were checked in supernatant of aliquots removed at regular intervals of 24 hrs .Growth was checked for ability of isolate to survive in presence of acetonitrile and its degraded products. Ammonia determination was done by Nesslerization spectrophotometric method Here brown colour produced by the reaction of ammonia with nessler's reagent was determined calorimetrically at 425 nm.[21]. A set of standards was run using ammonium chloride as standard, and the range was 1-5mcg/mlConcentration of ammonia released in aliquots that were taken every 24 hrs ,was determined from standard graph [22]

3. RESULTS

Sampling and Isolation

A total of 45 samples comprising of soil, water and sewage were collected from Ulhas river basin.The result for the same is presented in figure 1. From these samples 85 probable *Arthrobater* isolates were obtained, as also depicted in figure.2. These isolates were maintained on respective slants and were refrigerated till further studies.

Identification

Gram staining and morphological studies: 85 isolates showing typical glistening drop like colony (characteristic of *Arthrobacter*) were taken for studying morphological characteristics. All the isolates showed gram variable mixed rods and cocci morphology under microscope. Subsequently morphogenesis by Phase Contrast Microscopy (PCM) was also carried out. A typical *Arthrobacter* culture should also show rod to coccus morphogenesis within few hours. Hence the observations were made at every 0, 2 and 24 hours under Phase contrast microscope. Images recorded with PCM confirmed the rod-to-coccus morphology of *Arthrobacter* isolates (Figure 3).Sixteen isolates were segregated out on the basis cumulative observations presented in table 2.1

Morphogenesis i.e; rod-to-coccus of all the probable *Arthrobacter* isolates wass observed to be matching with that of standards. Only isolate no.A18 & A22 showed lesser number of differentiating cells.

Biochemical tests: of the above segregated isolates were also performed. The results of biochemical tests of the probable *Arthrobacter* isolates are shown in table 2.2.

All the probable *Arthrobacter* isolates were gelatinase positive, an, essential biochemical test of *Arthrobacter* and were also catalase positive The isolates showed glucose degradation producing acid but no gas. The isolates were cellulose negative and oxidase negative except A11,A13,A27& A40. Only isolate no.s A40 and A51 showed oxidative-fermentative test positive.

Biodegradative Profile/Screening. The qualitative biodegradation studies were performed by incorporating the pollutant/compound in different concentrations in respective agarified medium with the compound to be degraded as sole source of carbon. The results were observed as growth/no growth of the selected isolates, in presence of respective pollutant/compound at 24- 48 hours of incubation at R.T. The results of these degradations are given in different tables, mentioned below.

Table No.3.1 depicts degradation of Organic compounds. All 16 selected isolates could grow in presence of ethyl acetate and benzene, but not in presence of phenol and Nicotinic acid, except one isolate A72 which showed growth in presence of nicotinic acid. 50 % and 56 % of isolates could grow in presence of xylene and toluene respectively.

Table No 3.2 shows degradation of Nitrogen containing Organic compounds.There was not a single isolate which could not grow in presence of Acetonitrile and TMA(trimethylaniline). 75%and 50%of the isolates could grow in presence of Picoline and Aniline respectively .Very few i.e; only 38% isolates could grow in presence of DPA(Diphenylamine) .

Table No 3.3 represents degradation of Dyes.All the selected isolates could grow in presence of Methyl orange(MO).OnlyA76&A83 could not grow in presence of Methyl green(MG). 38% of the isolates could not grow in presence of Methyl blue(MB). Only 25% of isolates could not grow in presence of Methyl red (MR) dye.Overall maximum number of isolates could grow in presence of dyes.

Table No 3.4 illustrates degradation of Lignocellulosic wastes. All the selected isolates could grow in presence of sawdust. Very few isolates i.e; about 25% and 31% could not grow in presence of Xylan and Carboxymethyl cellulose(CMC) respectively . However 63% isolates could grow in presence Lignin .

Table No 3.5 outlines degradation of . Inorganic compounds& Organochlorines.All the selected isolates could grow in presence of Chromium nitrate except A13,A51,A72&AS1.About 75% isolates could grow in presence of Chromium as dichromate.Only 19%isolates could not grow in presence of Mercuric chloride. Overall isolates appear to be good heavy metal degradors.Also 63% isolates could grow in presence of Organochlorine i.e;Pentachlorophenol(PCP).

Table no.3.6 describes the overall biodegradative profile of the isolates.

Isolates nos A3, A11, A18 & AS1 couldgrow in presence of maximum number of compoundsi.e;18-19

Isolate no.A1,A2,A22,A46 & A72 also grow in presence of an 15-17 compounds.

Remaining isolates could degrade about minimum 7-15compounds.

Figure no.4 is the graphical representation of biodegradative profile depicted in Table no.3.6.Name of isolates on X-axis and No. of compounds degraded on Y-axis

Quantitative degradative analysis

Degradation/decolourisation of Methyl orange. The degradation/decolourisation of methyl orange was studied and the results of percentage decolourisation are given in table no 1.1.Figures 5,6 and 7 show decolourisation at 0, 24 and 48 hours.

As can be seen from the table , 7 isolates showed decolorization of 70 to 95% when incubated for 48hrs. The other 5 isolates too showed 60 to 70 % decolourisation. Standard culture also shows decolorization upto 89%

Degradation of inorganic compound--Chromium**.**

Degradation of inorganic pollutant/compound chromium was studied and the results of percentage degradation are depicted in Table No.2.1. Figure No.8 depicts the standard graph of Chromium

As shown in Table No.2.1 around 75% degradation was shown by isolate No. A51 & A83 The rest of the isolates too showed degradation, but in the range of 40 to 70%.

Degradation of organic compound --Acetonitrile. Degradation of acetonitrile by potent isolates and standards was studied. The results of percentage degradation and growth measured are depicted in Table No.3.1 & 3.2 respectively. The standard graph of ammonia is shown in Fig 9.

As outlined in below tables three isolates showed 73-80 % along with standard.All other isolates show degradation in range of 26-68%. Almost all the isolates grew very well in medium with acetonitrile as sole source of carbon,in 48hrs..

4. CONCLUSIONS

The requirement of modern times is to develop microbial inocula for bioremediation of contaminated niches such as soils and polluted waters. The natural attenuation potential of these sites needs to be assessed..Not only it is necessary to know the degrading abilities of native microbial populations but also their persistence and activity needs to be considered [23] *Arthrobacter* is a common occurant in these habitats.It is reported to have remarkable ability to degrade a wide range

of compounds as well as survive under such stressed environments[4,5].The above project was an effort to isolate *Arthrobacter* strains from soil and sewage samples of Ulhas river basin.The isolates were identified by colony characteristics, gram staining, biochemical tests and morphology. Phase contrast microscopy validated typical rod-to coccus morphogenesis *of Arthrobacter*.16SrRNA gene analysis confirmed the identification . Standard cultures of *Arthobacter* were constantly used for comparison. Isolates were found to degrade a wide range of compounds such as organic pollutants, nitrogen containing organic pollutants,dyes,lignocellulosic wastes, inorganic pollutants such as chromium and organochlorines qualitatively.The possible potent isolates were also assessed for their quantitative biodegradative potential .The potent *Arthrobacter* isolates were able to decolorize dye methyl orange upto 95%, degrade inorganic pollutant chromium upto 75% and organic pollutant acetonitrile upto 80%..Thus observing the qualitative as well as quantitative biodegradative profile and its ubiquitous presence in soil, one could explore *Arthrobacter* to remediate subsurface pollution and environmental cleanup by bioremediation. For practical application, one of the main challenges that needs to be overcome is establishment of technically and economically feasible bioremediation strategies[23] .

ACKNOWLEDGEMENTS

We are thankful to our principal for providing us with facilities and encouragement for carrying out this project at Smt.C.H.M College, Ulhasnagar.

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List of Figures and Tables:

Figure. No. 1 Sampling Data

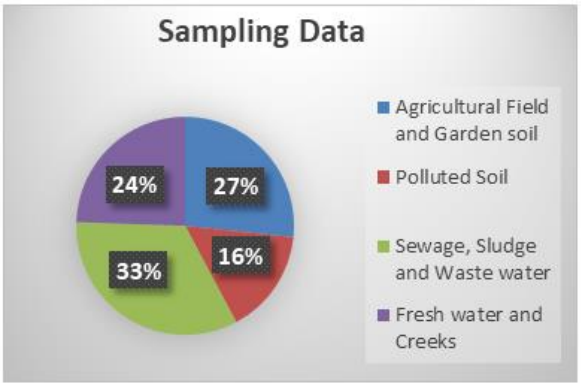


Figure No.2.Isolation of *Arthrobacter*. from samples

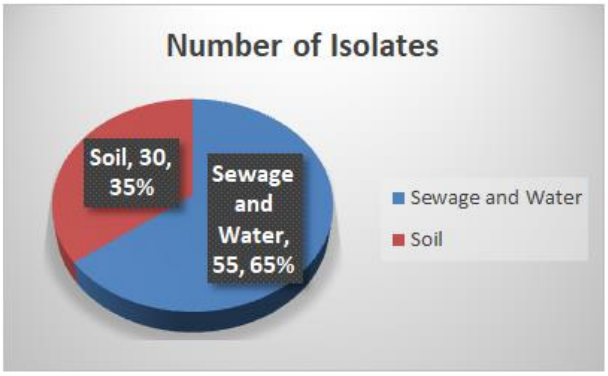
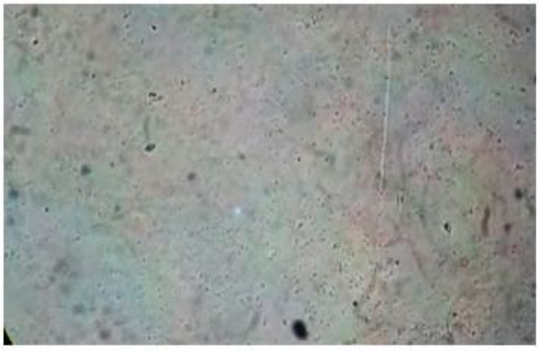
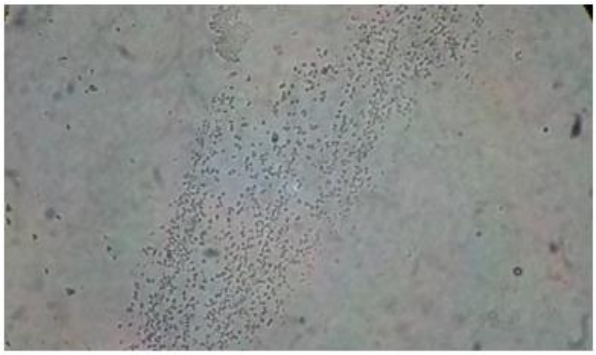


Figure No. 3 Isolates showing typical rod to coccusmorphology under PCM





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Vol. 7, Issue 2, pp: (481-494), Month: April - June 2019, Available at: www.researchpublish.com

Table 2.1 Morphogenesis under Phase contrast Microscope

Isolate No	0 Hrs	2 Hrs	24 Hrs
A1	+	++	++
A2	+	++	++
A3	+	+	++
A11	+	++	++
A13	+	++	++
A15	+	++	+
A18	+	+	+
A22	+	+	+
A27	+	++	++
A40	+	++	++
A46	+	++	++
A-51	+	++	++
A71	+	++	++
A72	+	++	++
A76	+	++	++
A83	+	++	++
Standards(S1&S2)	++	++	++

Table 2.2. Biochemical tests of probable *Arthrobacter isolates*

Isolate no	Glucose	Catalase	Gelatinase	Cellulose	OF	Oxidase
A1	+	+	+	-	-	+
A2	+	+	+	-	-	+
A3	+	+	+	-	-	+
A11	+	+	+	-	-	-
A13	+	+	+	-	-	-
A15	+	+	+	-	-	+
A18	+	+	+	-	-	-
A22	+	+	+	-	-	+
A27	+	+	+	-	-	-
A40	+	+	+	-	+	-
A46	+	+	+	-	-	+
A51	+	+	+	-	+	+
A71	+	+	+	-	-	+
A72	+	+	+	-	-	-
A76	+	+	+	-	-	+
A83	+	+	+	-	-	+
S1	+	+	+	-	+	+
S2	+	+	+	-	+	+

Morphogenesis(rod to coccus (+) No Morphogenesis (-) for Table No.2.1

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Table No.3.1 Organic compounds						
Compound Isolate No	Xylene	Toluene	Ethyl acetate	Benzene	Phenol	Nicotinic Acid
A1	+	-	+	+	-	-
A2	-	+	+	+	-	-
A3	-	+	+	+	-	-
A11	-	+	+	+	-	-
A13	+	-	+	+	-	-
A15	+	-	+	+	-	-
A18	+	+	+	+	-	-
A22	+	+	+	+	-	-
A27	-	+	+	+	-	-
A40	-	+	+	+	-	-
A46	-	+	+	+	-	-
A51	-	-	+	+	-	-
A71	+	+	+	+	-	-
A72	-	-	+	+	-	+
A76	+	-	+	+	-	-
A83	+	-	+	+	-	-
AS1	+	+	+	+	-	-
AS2	-	+	+	+	-	-

Table No. 3.2 Nitrogen containing Organic compounds

Compound Isolate No	Picoline	Acetonitrile	TMA	DPA	Aniline
A1	-	+	+	+	+
A2	-	+	+	+	+
A3	+	+	+	+	+
A11	+	+	+	+	-
A13	+	+	+	-	-
A15	-	+	+	-	-
A18	+	+	+	-	+
A22	+	+	+	+	-
A27	-	+	+	-	-
A40	+	+	+	-	+
A46	+	+	+	-	+
A51	-	+	+	+	-
A71	+	+	+	-	+
A72	+	+	+	-	-
A76	+	+	+	-	+
A83	+	+	+	-	+
AS1	+	+	+	-	-
AS2	-	+	+	-	-

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 2, pp: (481-494), Month: April - June 2019, Available at: www.researchpublish.com

Table No 3.3 Dyes

Compound Isolate No	MO	MG	MB	MR
A1	+	+	-	+
A2	+	+	+	+
A3	+	+	+	+
A11	+	+	+	+
A13	+	+	+	+
A15	+	+	+	+
A18	+	+	+	+
A22	+	+	+	-
A27	+	+	-	+
A40	+	+	-	+
A46	+	+	+	+
A51	+	-	-	-
A71	+	+	+	+
A72	+	+	+	+
A76	+	-	-	-
A83	+	-	-	-
AS1	+	+	+	+
AS2	+	+	+	+

Table No 3.4 Lignocellulosic waste

Compound Isolate No	Lignin	Xylan	Carboxymethyl cellulose	Sawdust
A1	+	+	+	+
A2	-	+	+	+
A3	+	+	-	+
A11	-	+	+	+
A13	-	+	+	+
A15	-	+	+	+
A18	-	+	+	+
A22	-	+	-	+
A27	+	-	-	+
A40	-	+	-	+
A46	-	+	+	+
A51	+	-	-	+
A71	-	-	+	+
A72	+	+	+	+
A76	-	+	+	+
A83	-	-	+	+
AS1	+	+	-	+
AS2	-	-	-	+

ISSN 2348-313X (Print)
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Table No.3.5. Inorganic compounds and Organochlorine

Compound Isolate No	Chromium nitrate	Cr as dichromate	Hg as Hg Chloride	PCP
A1	+	-	+	-
A2	+	-	+	-
A3	+	+	+	+
A11	+	+	+	+
A13	-	-	+	+
A15	+	+	+	-
A18	+	+	+	+
A22	+	+	+	+
A27	+	+	+	-
A40	+	+	+	-
A46	+	+	+	-
A51	-	+	+	-
A71	+	+	-	-
A72	-	-	+	-
A76	+	+	-	+
A83	+	+	-	-
AS1	-	+	+	+
AS2	+	+	-	-

Table No 3.6 Biodegradative profile of the isolates

Isolate No	No. of compounds degraded(Out of 19)
A1	16
A2	16
A3	18
A11	18
A13	15
A15	13
A18	19
A22	17
A27	13
A40	11
A46	17
A51	12
A71	15
A72	16
A76	13
A83	12
S1	19
S2	7

+ Growth - No Growth

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International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 2, pp: (481-494), Month: April - June 2019, Available at: www.researchpublish.com

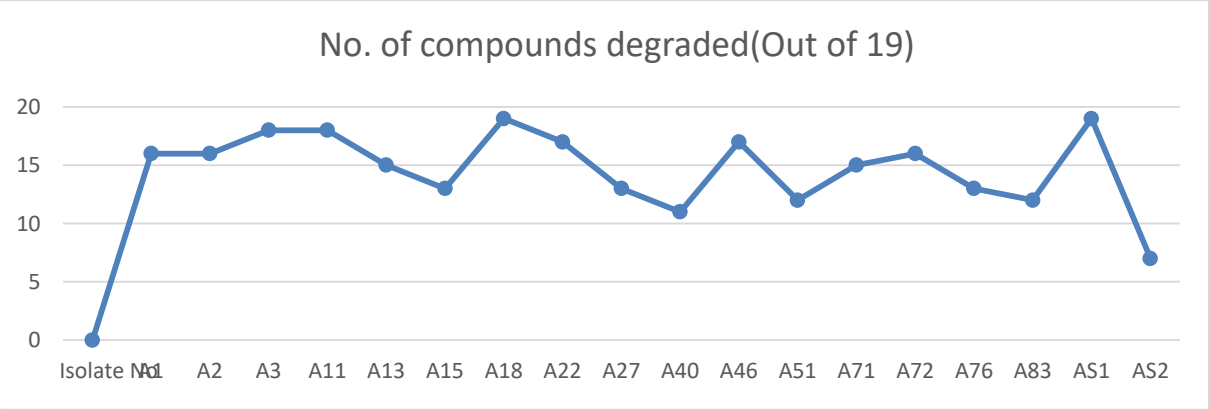
Table No.4.1 . %age Decolourization of dye

Isolate no	24hrs	48hrs
A1	62.18	64.65
A2	86	80.9
A3	12	69.7
A11	61.78	62.6
A15	60.08	67.9
A22	60.55	66
A40	89.4	88.3
A46	70.1	71.1
A51	92	93.4
A71	78.9	71.6
A76	59.6	72.6
A83	32.77	72.5
AS1	74.7	48.3
AS2	96	89.3

Table No.4.2 Percentage Degradation Hexavalent Cr^{+6} to trivalent Cr^{+3}

Isolate no	**24 hrs**	**48hrs**
A1	18.53	20
A2	36.73	51.75
A3	20.66	40.66
A11	27.5	53.6
A15	44	40.66
A22	47.72	53.6
A40	58.73	58
A46	27.5	51.8
A51	68	73.8
A71	18.53	48.06
A76	70	55.4
A83	55.06	75.76
S1	23.86	31.33
S2	40.38	81.30

Figure. No.4. Biodegradative profile of the isolates



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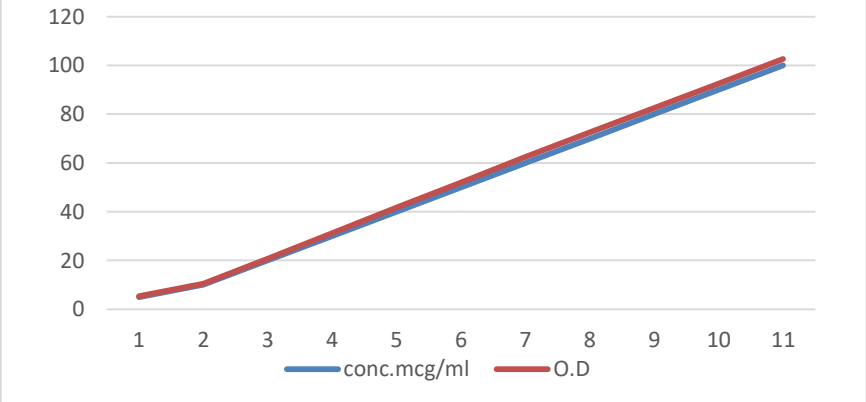
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 2, pp: (481-494), Month: April - June 2019, Available at: www.researchpublish.com

Figure No.5 Decolorization at 0 hrs

Figure No.6 Decolorization at 24 hrs

Figure No.7 Decolorization at 48 hrs

Figure No.8.Standard Chromium graph



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Vol. 7, Issue 2, pp: (481-494), Month: April - June 2019, Available at: www.researchpublish.com

Figure No. 9. Standard graph for Ammonia

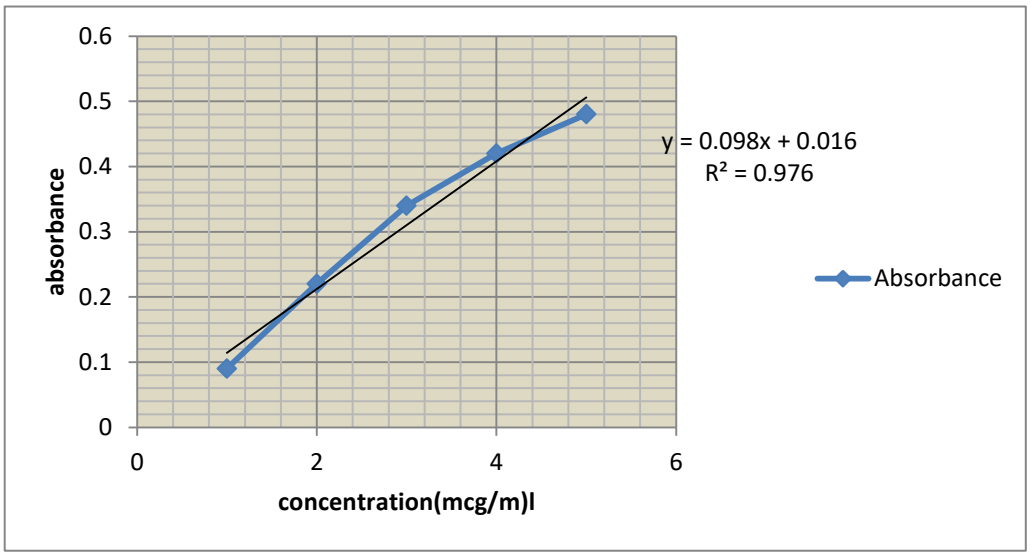

Table No.4.3 Percentage degradation of Acetonitrile

Isolate no	24 hrs	48 hrs
A 1	44.7	59.90
A2	33.53	58.9
A3	26.34	56.90
A11	73.17	78.24
A15	40.64	68.08
A22	5.07	30.48
A40	41.65	62.92
A46	41.65	59.95
A51	46.74	49.79
A71	52.83	80.28
A76	48.78	73.17
A83	40.58	67
S1	37.6	70.11
S2	30.48	52.78

Table No.4.4 Growth in Acetonitrile

Isolate no	Optical density (660)	
	24 hrs	48hrs
A1	0.04	0.07
A2	0.11	0.08
A3	0.02	0.06
A11	0.06	0.12
A15	0.12	0.14
A22	0.04	0.07
A40	0.12	0.13
A46	0.11	0.15
A51	0.06	0.08
A71	0.08	0.14
A76	0.05	0.11
A83	0.08	0.09
S1	0.06	0.08