*International Research Journal of Environment Sciences*_____ ISSN 2319–1414
Vol. **2(10),** 14-21, October **(2013)** *Int. Res. J. Environment Sci.*

Optimization and Characterization of Indigenous Microorganisms Isolated from Tannery Effluents in Nigeria

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Available online at: www.isca.in
Received 22nd June 2013, revised 19th July 2013, accepted 26th August 2013

Abstract

Samples of tannery effluents collected at the end of waste discharge pipes of tanneries in Kano, Nigeria were analysed to isolate, identify and characterize the indigenous bacteria and fungi capable of highly utilizing the tannery effluent as the sole carbon source. On the basis of morphological and biochemical characterization, the bacterial isolates identified were Klebsiella aerogenes, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis and Proteus vulgaris. The fungal isolates were Penicillium chrysogenum and Aspergillus niger. All the isolates had proliferated growth on Tannery Wastewater Agar (TWA). The bacterial isolates had 75-100% occurrence in the effluent samples which the fungal isolates were found preent in all the samples. The bacterial isolates had their optimal growth at temperature of 37^0C, pH 7, 20% tannin concentration, 20% $((NH_4)_2SO_4)$ concentration and 5% inorganic nitrogen source. The optimal growth for the fungal isolates was achieved at temperature of 28^0C, pH 4.5, inorganic and organic nitrogen concentrations of 20% and 15% respectively and at 20% tannin concentration. All the isolates grew better in the inorganic source of nitrogen $((NH_4)_2SO_4)$ than in the organic source (meat extract). The results revealed that the isolates were able to utilize the tannin hence the ability to grow well at the high concentration. They can thus be recommended for use in the biodegradation of tannin which is one of the major toxic constituents of tannery effluent.

Keywords: Tannery, effluents, isolates, waste bacteria, fungi, indigenous.

Introduction

Tanning is the treatment of hides and skins to preserve and convert them into a stable material (leather) which will not putrefy and is suitable for a wide variety of applications. The steps in the conversion process are grouped into beam house and tanyarrd operations. The beamhouse is where the preparatory processes for tanning ensue while the tanyard is actually where the leathers are produced[1]. All these processes occur in the tannery. The tanning processes generate wastes that are of serious environmental impact particularly in countries where environmental regulation are lax. The unused and non-usable hides and skins along with the excess process chemical and water constitute solid and liquid wastes in the tannery[2]. These wastes when untreated affect streams, groundwater, land and sewers in which they are discharged. This discharge of untreated wastewater in water courses affect the physical, chemical and biological characteristics of water and deplete the water bodies of dissolved oxygen[3-5].

Tannery waste is generated in huge amounts during the process of tanning by leather industries throughout the world. It is considered one of the most polluted industrial wastes and contains high amounts of metals which are very toxic to plants, animals and soil[6]. Less than 5% of the industries in the world have adopted the adequate measure for treatment of effluent while most have neglected it because of cost implications[7-9]. When tannery wastes gain access to cultivable lands or when the lands are irrigated with such wastes, the fertility of the soil is affected[10].

Tannery wastes are known to reduce germination, growth and yield of grain, wheat and lettuce crops[11,12]. It has also been reported that plant uptake metals such as chromium from tannery wastes and become available in roots, shoots, leaves, flowers and fruits with the minimum in fruits[13]. The diverse biochemical nature of microorganisms make it possible for them to metabolise most organic compounds found in industrial wastes hence constitute the basic biological units in tannery effluent treatment system.

Microorganisms have been reported to remove some of the waste constituents. Yang and Lee[14] degraded phenol completely within 57.5 hours and 93.1hours using *Pseudomonas resinovorans* and *Brevibacillus sp.,* respectively. Naresh[15] biodegraded phenol using a bacterial strain isolated from a phenol contaminated site. Murugan[16] isolated *Aspergillus niger* capable of producing tannase which was able to degrade tannin, a major constituent of tannery effluent. Nachiya and Rajkimar[17] decolorized navatan fast blue (an important commercial diazo dye in the tanning and textile industries) using *P. aeruginosa.* Mir[18] reported that dye from industrial effluent affected the physicochemical properties of soil. Microbial biochemical processes play useful roles in the safe disposal of tannery waste. These processes are influenced by pH, temperature, nutrient availability, temperature, biomass behavior and concentration[19].

Organisms in the tannery effluents have been reported to adopt measures that enable them tolerate and utilize the hazardous constituent of tannery effluents. Such measures include acquisition of plasma[20]. Indigenous organisms thus are well adapted for the treatment of tannery effluent.

In a bid to use indigenous organisms that have been well adapted to the constituents of tannery effluent, it is imperative that optimum conditions that will enhance their ability to detoxify the effluent be investigated. The knowledge of these conditions which will enhance their optimal growth will provide information on the conditions that will make them function optimally for the elimination of the toxic components of tannery effluent.

This study therefore is aimed at isolating and identifying microorganisms associated with tannery effluents in Nigeria and determining the optimal growth conditions for selected isolates able to utilize tannery wastewater effectively.

Material and Methods

Collection of samples: Samples of effluents from four tanneries in Kano, Nigeria were collected at the end of the waste pipes from which they are discharged. They were collected in plastic containers and transported in ice-packs to the laboratory where they were kept in the refrigerator at 4^0C.

Isolation and identification of organisms: An aliquot of 01.ml of the appropriate dilution of serially diluted samples was spread unto Nutrient Agar plates (for bacterial isolates) and Potato Dextrose Agar plates (for fungal isolates and incubated at 28 $\pm 2^0$C for 24 and 48 hours respectively. District colonies were subcultured for purity and transferred to agar slants and stored in the refrigerator at 4^0C for future use. District colonies on each plate were counted at the end of incubation.

Growth of Isolates in Tannery Waste Water Medium: For this purpose, Tannery Wastewater Agar (TWA) was used as the sole carbon source. The method of Prakasam and Dondero [21] was used in preparing TWA. The tannery wastewater (IL) was autoclaved at 121^0C for 30 minutes before filtering through glasswool. The filtrate was made up to litre with freshly distilled water incorporated with mineral salts (g/l: K_2HPO_4 (0.5), $MgSO_4.7H_2O$ (0.01), $(NH_4)_2\ SO_4$(1.0), K_2HPO_4 (1.3), KCL (0.05), $FeSO_4.7H_2O$(0.01). Thereafter 15g of agar powder (oxoid) was added for solidification. This was autoclaved again for thirty minutes. On cooling, the TWA plates were inoculated with the test isolates and incubated at 28^0C$\pm 2^0$C for 24hours and 48hours for bacteria and fungi respectively.

Appearance of colonies on the plates indicated that the isolates were capable of growth in Tannery wastewater.

Identification of Isolates: The identification of the bacterial isolates was done using the morphological characteristics (Shape, gram reaction, motility and cultural characteristics) and biochemical characterization. Fungal isolates were identified mounted on lactophenol and viewed under the microscope. The final identification of the isolates was done following Bergey's Mamal of Determinative Bacteriology[22] for bacterial isolates and keys of Barnett[23] for fungal isolates.

Optimization Tests: Each isolate was inoculated into culture media varying pH, temperature, Nitrogen sources and tannin concentrations. The media were then incubated at 28 ± 2^0C for bacterial and fungal isolates for 24 and 48 hours respectively.

Effect of organic Nitrogen: Meat (500g) was boiled for about twenty minutes. It was allowed to cool and sieved to get the meat extract. 5%, 10%, 15% and 20% (v/v) of the meat extract was introduced into the mineral salt medium from which $(NH_4)_2SO_4$ had been omitted.

Inorganic Nitrogen: 5%, 10%, 15% and 20% (w/v) of $(NH_4)_2SO_4$ dissolved in sterile distilled water was introduced into the mineral salt medium.

Tannin: The effect of tannin on the growth of the isolates was done using 5%, 10%, 15% and 20% (w/v) tannin powder dissolved in distilled water and then introduced into the mineral salt medium.

pH: The growth of each isolate was monitored by incubating each isolate under optimum concentrations of nitrogen and tannin and at pH values 4.5, 7 and 8.5.

The mineral salt medium was made up to 1L with distilled water and sterilized at 121^0C for 30 minutes. Each isolate was inoculated into the sterilized medium and incubated at 28 ± 20^0C for 24 and 48hours for bacteria and fungi respectively.

Microbial growth in culture tubes was determined spectrophotometrically by measuring absorbance at wavelength 600nm with UV-visible spectrophotometer.

Results and Discussion

The bacteria isolated from the tannery effluent on the basis of biochemical and morphological characterization were *klebsiellla aerogenes, Escherichia coli, Pseudomonas aeruginosa, Protens vulgaris, Bacillus subtilis, Staphylococcus aureus* and *Micrococcus roseus.* The fungal isolates were *Aspergillus niger, Paecilomyces variotii* and *Saccharomyces cerevisae* (table 2).

K. aerogenes, B. subtilis, P. chrysogenum and *A. niger* were found to be present in all the effluent samples while *P. aeruginosa, E. coli* and *P. vulgaris* had 75% occurrence, *P variotii* and *S. cerevisae* had the least occurrence of 60% (table 3).

Mohammed[24] isolated similar bacteria with those isolated in the study from tannery effluent but reported that *Pseudomonas* and *Bacillus spp*. were the major isolates and *Klebsiella sp* was

found to be subdominant. Mathuprakash and Jayanthi [25] Isolated *Aeromonas*, *Alcaligenies*, *Staphylococcus* and *Pseudomonas* from tannery effluents. The utilizable nutrients such as Nitrogen, Phosphorus in the effluents are made use of for the growth of the organisms. Most of the organisms in the tannery effluent utilize phenol and other pollutants as their carbon source to produce metabolic energy [26]. Comparison by t-test showed that growth was generally less in tannery wastewater (TWA) than on Nutrient agar (table 4).

Table-1
Microbial Population in Effluent (CFU/ml)

Sample	Bacteria					Fungi				
	Nutrient agar (NA)		Tannery Wastewater Agar (TWA)		P. Sign	Saboraud Dextrose Agar		TWA		P. Sign
	Mean	STDV	Mean	STDV		Mean	STDV	Mean	STDEV	
1	13.62	0.14	9.91	0.07	<0.05	10.29	2.09	6.36	0.15	<0.05
2	12.10	0.01	9.84	0.25	<0.05	13.51	0.12	6.40	0.13	<0.05
3	12.16	0.06	9.83	0.16	<0.05	13.87	0.05	6.53	0.09	<0.05
4	12.01	0.12	9.92	0.20	<0.05	7.42	0.19	6.35	0.14	<0.05
				P =	**0.003**				**P =**	**0.023**

Table-2
Characterization and Identification of Isolates from the tannery effluent of water and soil samples contaminated with tanning effluent

Morphological type	Gram Reaction	Catalase	Maionate	Citrateutilization	Motility	Aerobic growth	anaerobic test	mannitol acid	Voges Proskaver	methy/Red	Lysine Decarboxyle	Arginine decarboxylase	Ornithine Decarboxylase	Nitrate Redcution	Galatine Liquefation	Urease	Indole	H25 Production (TSI)	Lactose (Acid Production)	Sucrose (Acid)	Caogulase	Glucose (Acid)	Identity
C	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	K. aerogenes
r	-	+	-	-	+	+	-	+	-	+	+	-	+	-	-	-	+	-	+	+	-	+	E. coli
r	-	+	-	-	+	+	+	-	-	-	-	+	-	-	+	+	+	-	-	-	+	+	P. aeruginosa
r	-	+	-	-	+	+	-	+	-	-	-	-	-	-	-	+	+	-	-	-	+	+	P. vulgaris
r	+	+	-	-	+	+	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	B. subtilis
C	+	+	-	-	-	+	-	+	-	+	-	-	-	+	-			-	+	+	+	+	S. aureus
C	+	+	-	-	+	+	-	-	-	+	-	-	-	-	-	+	-	-	+	+	-	-	Micrococcus
Key:	_ = negative, + positive																						

Table-3
Occurrence of Isolates in the Tannery effluent water and soil sample

Sources	P. aeruginosa	K. aerogenes	E. coli	Bacillus subtilis	Proteus vulgaris	P. chrysogenum	A. niger	P. varioti	S. cerevisae
Soil Sample 1	-	+	+	+	++	+	+	+	-
Soil Sample 2	+	+	-	+	+	+	+	+	-
Soil Sample 3	+	+	+	+	++	+	+	-	+
Soil Sample 4	+	+	+	+	-	++	+	-	+
% Occurrence	75%	100%	75%	100%	75%	100%	100%	60%	60%

Key: + Present, - absent

The bacterial populations of the samples were higher on Nutrient Agar than on tannery wastewater Agar though they both encouraged good growths of the organisms. The better growth on Nutrient Agar might be attributed to presence of toxic substances in the tannery wastewater which may have inhibited the growth of some of the organisms thus contributing to the reduction on the microbial population in the tannery wastewater. The good growth on tannery wastewater containing toxic substances which otherwise should have inhibited the growth of microorganisms can be attributed to the ability of the organisms to acquire a variety of adaptations to the presence of some of the toxic components of the tannery effluent[27]. Zahoor and Rehman[28] reported that bacteria, yeast, algae, protozoa and fungi found in effluents have developed capabilities to protect them from the chemical constituents and heavy metals present in the effluents. The high population of bacteria (table 1) in the effluents might also be due to the inherent facility of many bacteria to form spores possessing tough outer-covering which facilitate their survival. The pH, temperature, water content and many other parameters especially availability of organic substrates in the effluent account for the microbial load and diversity[29]. The organisms isolated may have been introduced into the effluents from some various stages in leather manufacturing.

The differences in the percentage occurrence (table 3) of each isolate may be attributed to the precautions taken at each stage of production to eliminate or prevent entry of organisms. Yapici[30] reported that *E. coli* and other organisms which normally reside in the intestine of animal find their way into hides and skins during slaughtering. Orlita[31] also reported that the soaking liquor provides optimum conditions for proteolytic bacteria to grow fast hence the growth of organisms such as *E. coli, P. aeruginosa, P chrysogenum* and *A. niger* which are eventually introduced into the tannery effluent and subsequently disposed of.

All the bacterial isolates grew well within the temperature range (28 – 40^{0}C) indicating they were mesophilic however *B. subtilis* was still able to thrive well at 45^{0}C. (figure 1). *Bacillus subtilis* had the optimum growth at 28^{0}C. All the other organisms had the optimum growth at 37^{0}C. The growth may be attributed to the enzymes being stable and optimally metabolically active at 37^{0}C. There was no significant difference in the growth of the fungal isolates at the various temperatures they were exposed to.

Table-4
Population of each isolates on NA and TWA (Cfu/ml)

	Nutrient agar (NA)		Saboraud Dextrose Agar		TWA	
Isolate	**Mean**	**STDV**	**Mean**	**STDV**	**Mean**	**STDV**
K. aerogenes	8.060	0.019			5.518	0.109
P. aeruginosa	7.875	0.108			6.949	0.032
E. coli	8.076	0.127			6.640	0.072
B. subtilis	7.775	0.130			6.259	0.310
P. vulganis	7.706	0.267			6.851	0.076
P. chrysogenum			7.418	0.052	6.311	0.076
A. niger			6.669	0.060	5.527	0.331

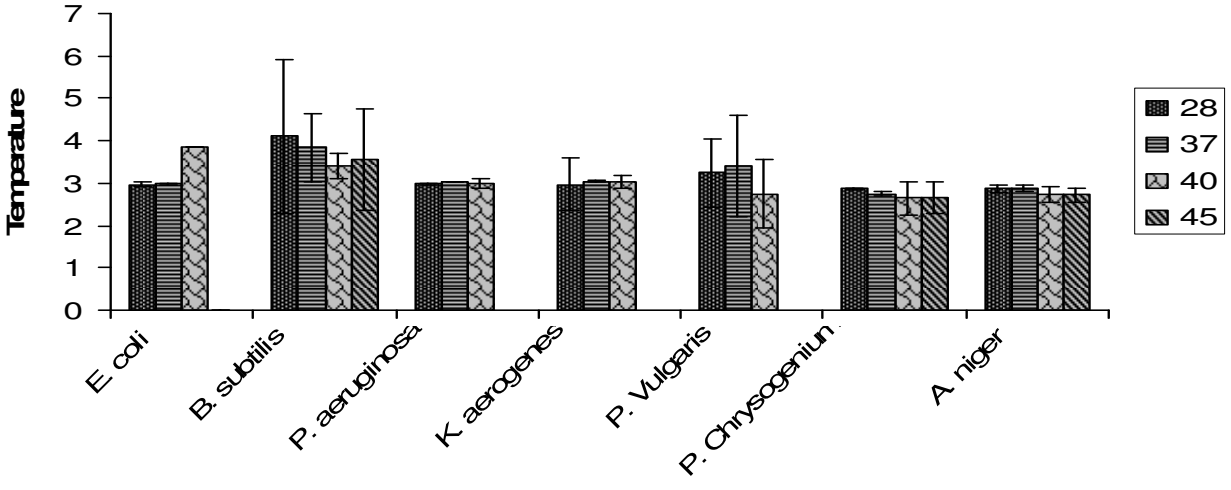

Figure-1
Turbidity measurement of the isolates at various temperature values at 450nm

All the bacterial isolates did not growth at pH 4.5 (figure 2) whereas the fungal isolates had their optimum growth at this pH value. There was no significant difference in the growths at 7.0 and 8.5 however *P. vulgaris* had the optimum growth at pH 7 which was significantly different from 8.5.

The organic nitrogen source enabled a good growth of all the isolates at the various concentrations. *K. aerogenes* had the optimum growth at 150% while *E. coli* and *P. aeruginosa* were at 10% *B. subtilis* at 10% and 20% and *P. vulgaris* at 10%. The optimum growth for *P. chrysogenum* was 5% while *A. niger* was at 20% (figure 3).

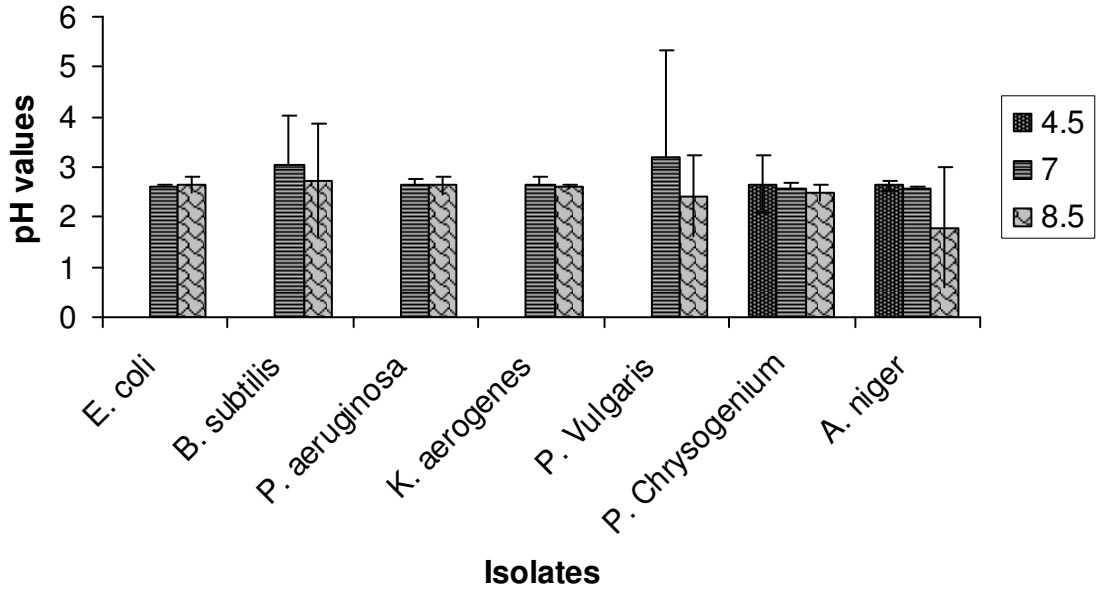

Figure-2
Turbidity measurement of isolates at various pH values at 450nm

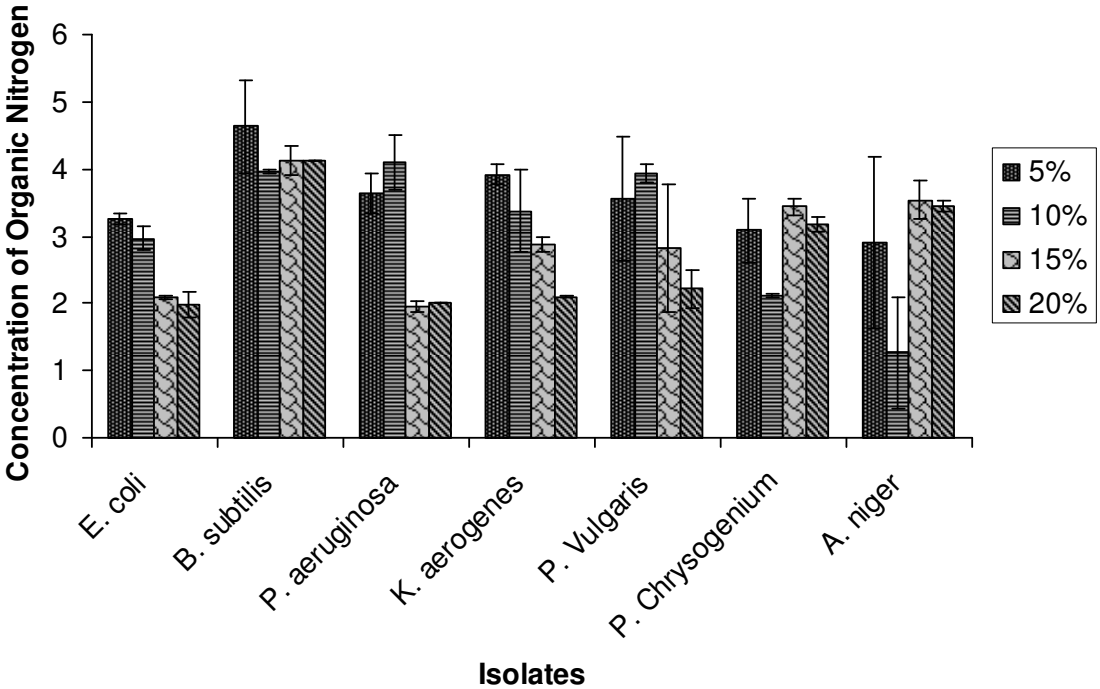

Figure-3
Turbidity measurement of the isolates in various concentration of organic nitrogen at 450nm

All the isolates had better growths in the inorganic source of nitrogen than in the organic source. All the isolates had their optimum growth at 20% concentrations of $(NH_4)_2SO_4$ and 20% tannin concentration. This indicates the ability of the organisms to degrade tannin hence the optimum growth at the high concentrations of tannin. Different types of microorganisms have been reported to utilize tannin as their sole carbon and energy source[32].

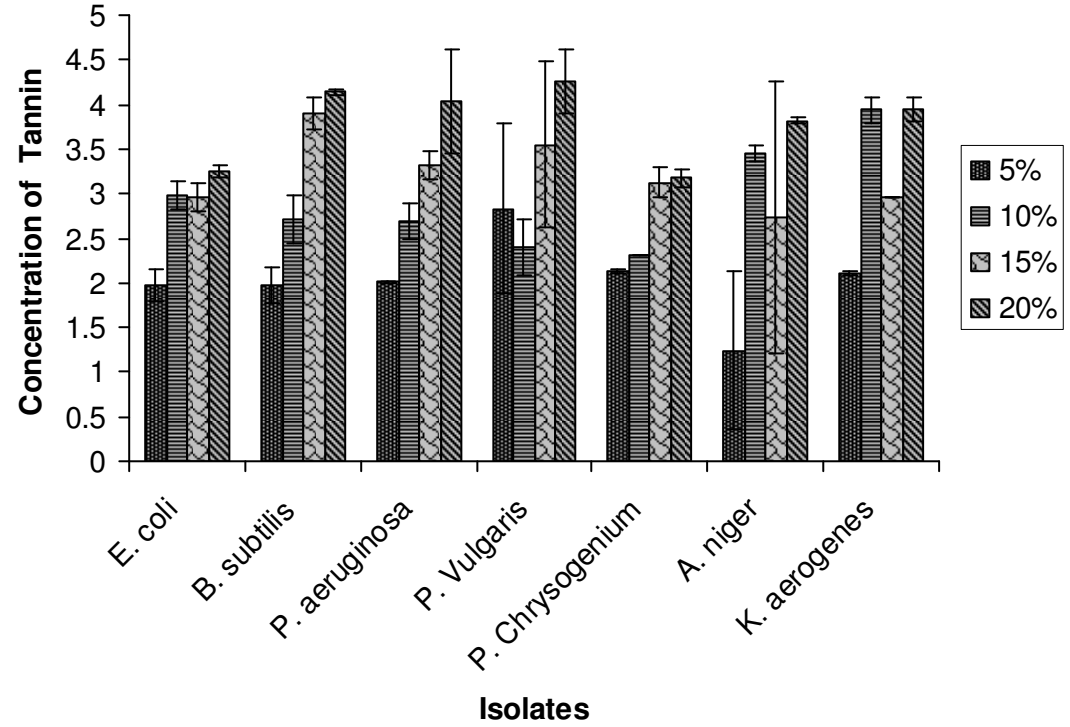

Figure-4
Turbidity measurement of isolates at various tannin concentrations at 450nm

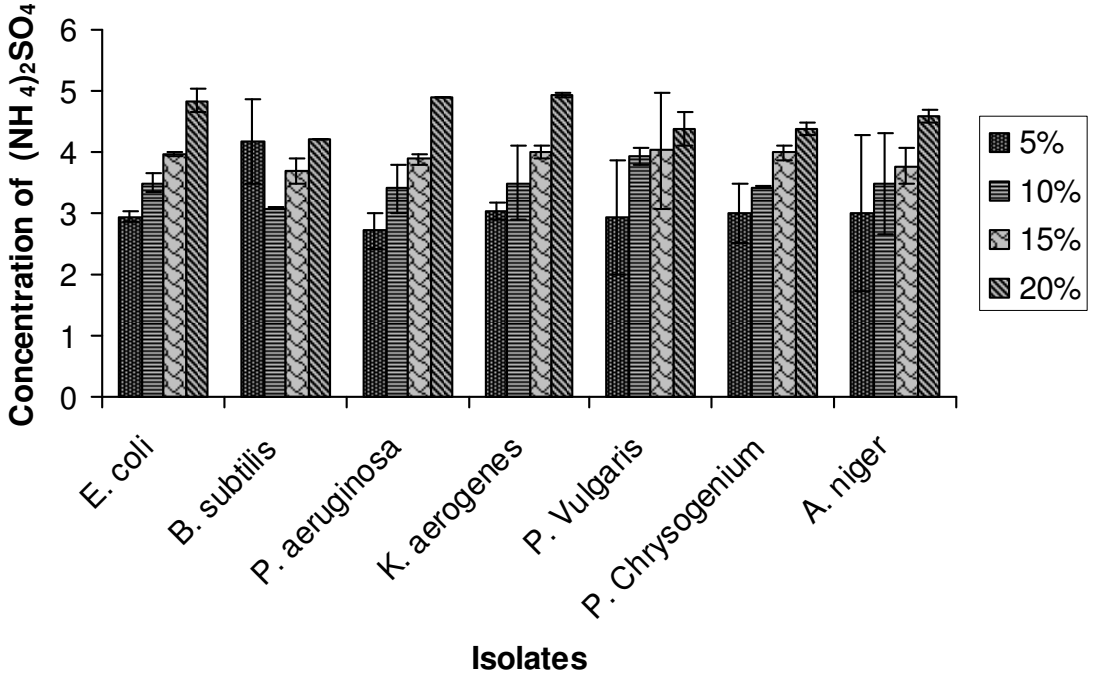

Figure-5
Turbidity measurement of isolates at various concentrations of $(NH_4)_2SO_4$ at 450nm

Conclusion

The isolates were mesophilic organisms and had optimal growth at pH 7 and 8.5 though the fungal isolates still grew well at pH 4.5. They had their optimum growth utilizing both the inorganic nitrogen source ($(NH_4)_2SO_4$) and tannin at 20% concentration. The isolates can be employed in the detoxification of tannery effluent suice they were able to grow optimally at the high tannin concentration (20%) which is a major component of tannery effluent. The optimization tests have provided information on amendment of the microbial environment in order to make it optimal for their growth and so enhance the tannery effluent detoxification potential of the isolates.

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