Optimization and biodegradation of chromium present in leather industrial effluents using indigenous microorganisms isolated from leather industrial sludge

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Abstract

Microorganisms Paracoccus pantotrophus (OP288256) and Bacillus velezensis (OP289289) are used as individual cultures and Co cultures in the biodegradation of Chromium, under different optimized conditions. Isolated microorganisms from Leather industrial sludge are used for the biodegradation of Chromium. The Amount of Chromium degradation individually by Paracoccus pantotrophus, Bacillus velezensis and Co cultures of P. pantotrophus+B.velezensis was observed at pH 7. There was a maximum degradation of chromium by P.pantotrophus, B.velezensis and P. pantotrophus+B.velezensis seen at temperature of 35°C. Chromium degradation by Paracoccus pantotrophus was higher in the media supplemented with Fructose as the carbon source, whereas Bacillus velezensis showed maximum chromium degradation in media that contained Glucose as the carbon source. Thus, Co cultures showed a significant amount of chromium degradation in media that used Glucose and Fructose as carbon source. A significant amount of chromium was degraded by P.pantotrophus in the media containing Yeast Extract as the nitrogen source, whereas degradation by Bacillus velezensis was higher in the media with Peptone and P.pantotrophus+B. velezensis showed a maximum degradation in the media with Glucose and Peptone as the Nitrogen source. More the concentration of the Inoculum added to the media, the amount of chromium degradation gradually increased by individual culture and Co cultures . Significant increase in the chromium degradation observed for the incubation from Day 7 to Day 28, by individual organism and combined cultures. Bioremediation using Co cultured bacteria is an economical and environmentally better alternative to conventional remediation methods.

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Keywords: Tannery Effluent (TE), Chromium Bioremediation, Pollution, Fructose, Chromium Bioremediation, Pollution, Fructose, TW15(Paracoccus pantotrophus), TW17(Bacillus velezensis)

1 Introduction

Industrial effluent for example leather industrial effluent contains heavy metals and other pollutants which affects the quality of water. The hexavalent chromium compounds are comparatively more toxic than trivalent chromium compounds due to the solubility in water, rapid permeability through biological membranes and subsequent interaction with intracellular proteins and nucleic acids (3).

Effluents from tanneries contain components of chromium and sulphides in most cases with a major proportion of dyes (9).Chromium are one of the most toxic elements present in tannery waste. High chromium concentrations ranging from 1 to 50g/kg were reported in soils surrounding tannery waste disposal sites in India with hexavalent chromium.

Most of the tanneries use chromium (Cr III and Cr IV) during tanning, which are highly toxic and

poses a severe threat to the environment upon improper disposal of their waste water. The less concentrations of these salts have a adverse effect on the food chain of fish and inhibits photosynthesis of aquatic plants (4). **(author?)** (6) reported heavy metal accumulation in vital human organs through the consumption of crops after the discharge of tannery effluents for irrigation purpose. This results in illnesses on acute and chronic exposure, such as cancer, kidney dysfunction, cholera and skin irritations (10).Heavy metals like Cr, Cu, Zn, Pb and Cd are mostly absorbed and get accumulated in different parts of the plants as free metals and it affects the plant growth and metabolism. Major diseases of cattle and human beings are caused by chromium and nickel (2).

Human when exposed to Cr(VI) they may suffer from several health hazards such as allergic dermatitis, nasal irritation, renal tubular necrosis, eardrum perforation ulceration, skin irritation, lung carcinoma, epidermal dermatitis and increase risk of cytotoxic and genotoxic effects (cell death, cell transformation and gene mutation) and respiratory tract cancer (11).

The regular treatment method of tannery wastes for the purpose of detoxification requires application of physical and chemical methods, which includes the chrome precipitation and filtration, specific coagulation, use of activated carbon and chemical flocculation (Olukanni et al., 2006). The contaminated water is treated using conventional methods for the purpose of

detoxification of toxic substances from the effluent. But due to associated problems in these treatment methods such as high cost, intense experimental set-up leading to post treatment effects (1), the alternative treatment methods can be used by using Bacteria, Fungi and Algae (Srinivas and Estari, 2013) because it is eco friendly and cost effective. Therefore, the aim of this study is to utilize bacterial cultures isolated from Leather industrial sludge and optimized various conditions for biodegradation of Cr .

2 Materials and methods

2.1 Isolation of strains

Serial dilution of the tannery effluent sludge was done and was plated on Peptone Yeast Extract(PYE) media supplemented with chromium (100g/ml) as potassium dichromate and also 0.5% glucose as a carbon source. The controls also kept without chromium and glucose. The plates were kept for incubation at 30ºC for 2 days. The pure colonies were maintained in PYE Agar media plates. (Das, A. P., 2010)

2.2 Determination of Chromium

The samples were taken in 3 tubes each containing 5ml of the test sample. Added 1ml of 1,5-diphenyl carbazide (DPC) reagent to all the tubes. Then, 1ml of 0.2N Sulphuric Acid was added to the tubes. After adjusting the volume of each tube to 7ml with distilled water, it was shaken and kept for incubation for 30 minutes at room temperature. Chromium was used as standard solution which was supplemented by potassium Chromate. The intensity of the color developed was measured at 540nm using UV-Visible spectrophotometer. The results of the chromium are expressed in terms of chromium present in mg/ml of extract. Distilled water is used as blanks (**Kefa et al , 2016**).

2.3 Optimization of pH

Minimal salt media with Potassium Dichromate in the concentration of 50mg/100ml was prepared. 100ml of media was transferred into 24 bottles each and adjusted the pH of the media as per the conditions- pH 3, 4, 5, 6, 7, 8, 9 and 10 and autoclaved. 3 bottles with the media was taken for each pH conditions and were inoculated with 5ml of TWI5 (Paracoccus pantotrophus), TW17 (Bacillus velezensis) and the consortium, TW15 (Paracoccus pantotrophus)+TW17(Bacillus velezensis) respectively and kept for incubation at 35°C for 7 days.

2.4 Optimization of Temperature

Minimal salt media with Potassium dichromate as the heavy metal in the concentration of 50mg/100ml was prepared. 100ml of media was transferred into 15 bottles each and autoclaved. 3 bottles with the media was taken for each temperature conditions and were inoculated with 5ml of TWI5(Paracoccus pantotrophus), TW17(Bacillus velezensis) and the consortium, TW15(Paracoccus pantotrophus)+TW17(Bacillus velezensis) respectively. The bottles then, were kept for incubation at 25°C, 30°C, 35°C, 40°C and 50°C for 7 days.

2.5 Optimization of Carbon Sources

Minimal salt media with various carbon sources containing Potassium Dichromate as the heavy metal in the concentration of 50mg/100ml was prepared. 100ml of media was transferred into 15 bottles each and autoclaved. 3 bottles with the media was taken for each carbon source and were inoculated with 5ml of TWI5 (Paracoccus pantotrophus), TW17 (Bacillus velezensis) and the consortium, TW15 (Paracoccus pantotrophus)+TW17(Bacillus velezensis) respectively. The bottles then, were kept for incubation at 35°C for 7 days.

2.6 Optimization of Nitrogen Sources

Minimal salt media with various nitrogen sources containing Potassium dichromate as the heavy metal in the concentration of 50mg/100ml was prepared. 100ml of media was transferre

2.7 Optimization of Inoculum Concentration

Minimal salt media with Potassium dichromate as the heavy metal in the concentration of 50mg/100ml was prepared. 100ml of media was transferred into 18 bottles each and autoclaved. 3 bottles with the media was taken for each inoculum concentrations and were inoculated with 0.50%, 1.00%, 2.00%, 3.00%, 4.00% and 5.00% of TWI5(Paracoccus pantotrophus), TW17(Bacillus velezensis) and the consortium, TW15(Paracoccus pantotrophus)+TW17(Bacillus velezensis) respectively. The bottles then, were kept for incubation at 35°C for 7 days.

2.8 Optimization of Chromium Concentration

Minimal salt media with various concentratios of Potassium dichromate as the heavy metal was prepared. The concentrations taken were 15mg/100ml, 25mg/100ml, 50mg/ml,

75mg/ml and 100mg/100ml. 100ml of media was transferred into 15 bottles each and autoclaved. 3 bottles with the media was taken for each concentrations and were inoculated with 5ml of TWI5(Paracoccus pantotrophus), TW17(Bacillus velezensis) and the consortium, TW15(Paracoccus pantotrophus)+TW17(Bacillus velezensis) respectively. The bottles then, were kept for incubation at 35°C for 7 days.

2.9 Optimization of Incubation Period

Minimal salt media with Potassium dichromate as the heavy metal in the concentration of 50mg/100ml was prepared. 100ml of media was transferred into 3 bottles each and autoclaved. 3 bottles with the media was taken and were inoculated with 5ml of TWI5(Paracoccus pantotrophus), TW17(Bacillus velezensis) and the consortium, TW15(Paracoccus pantotrophus)+TW17 respectively. The bottles then, were kept for incubation at 35°C and assay was performed for Day 7, Day 14, Day 21 and Day 28.

3 Results and Discussion

In this study, Bacterial isolates were isolated from leather industrial sludge and identified using biochemical and physiological characteristics of the two strains. The results are summarized in Table 1 and Figure 1

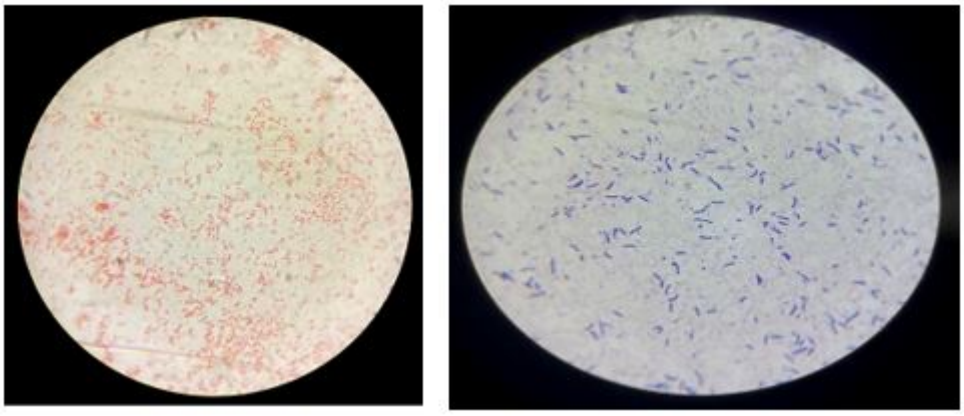

FIGURE 1

Bacteria stained pink Gram's-negative. Bacteria stained purple, Gram's-positive.

TABLE 1

Biochemical characterization of the strains

S.NO.	TEST	TW15	TW17
01.	CATALASE	-VE	-VE
02.	OXIDASE	+VE	+VE
03.	INDOLE	+VE	-VE
04.	METHYL RED	+VE	+VE
05.	VOGES-PROSKAEUR	-VE	-VE
06.	STARCH HYDROLYSIS	-VE	+VE
07.	SUGAR FERMENTATION		
08.	MANNITOL SALT AGAR	+VE	+VE
09.	CITRATE	+VE	-VE
10.	TRIPLE SUGAR IRON		
11.	UREASE	-VE	+VE
12.	NITRATE REDUCTION		
13.	BLOOD AGAR	-VE	+VE
14.	MOTILITY AGAR	-VE	-VE

3.1 Determination of Chromium

Concentration of chromium is determined colorimetrically using standard solution which is depicted in table 2 and Figure 2.

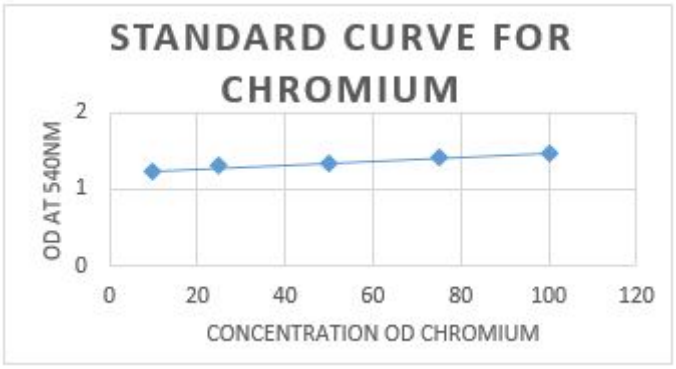

FIGURE 2

STANDARD Curvefor chromium

TABLE 2

Table 1 Determination of Chromiu

Conc.of Chromium (mg/100ml)	Absorbance at 540nm
10	1.223
25	1.303
50	1.342
75	1.408
100	1.466

3.2 Optimization of pH

Significant amount of Chromium degradation by TW15(Paracoccus pantotrophus), TW17(Bacillus velezensis) and TW15(Paracoccus pantotrophus)+TW17(Bacillus velezensis) was observed at pH 7 . The amount of chromium degradation seen at pH 5, 6 and pH 8 was lower but comparatively higher than that of pH 3, 4, 9 and 10.

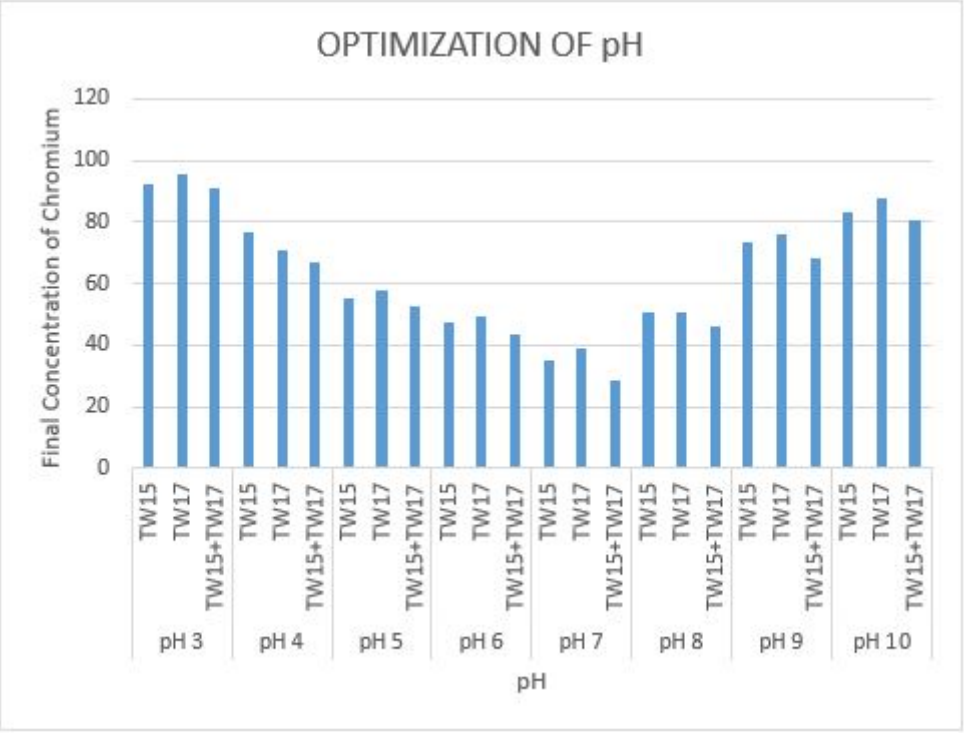

FIGURE 3

Chromium degradation by TW15,TW17and TW15+TW17 at optimum pH

Table 3

Chromium degradation by TW15, TW17 and TW15+TW17 at optimum pH

pH	ORGANISM	OD1	OD2	OD3	MEAN	STD DEV	Final Concentration of Chromium
pH 3	TW15	1.446	1.444	1.443	1.444333333	0.001527525	92.20555991
	TW17	1.453	1.457	1.455	1.455	0.001632993	95.78644218
	TW15+TW17	1.439	1.441	1.440	1.44	0.001	90.75082649
pH 4	TW15	1.399	1.397	1.397	1.397666667	0.001154701	76.53919998
	TW17	1.381	1.380	1.383	1.381333333	0.001527525	71.05597401
	TW15+TW17	1.368	1.370	1.371	1.369666667	0.001527525	67.13938402
pH 5	TW15	1.335	1.335	1.334	1.334666667	0.00057735	55.38961408
	TW17	1.341	1.342	1.342	1.341666667	0.00057735	57.73956807
	TW15+TW17	1.326	1.327	1.325	1.326	0.001	52.48014723
pH 6	TW15	1.310	1.312	1.309	1.310333333	0.001527525	47.2207264
	TW17	1.315	1.318	1.320	1.317666667	0.002516611	49.68258296
	TW15+TW17	1.295	1.299	1.301	1.298333333	0.00305505	43.19223385
pH 7	TW15	1.273	1.275	1.273	1.273666667	0.001154701	34.9114436
	TW17	1.287	1.286	1.284	1.285666667	0.001527525	38.93993615
	TW15+TW17	1.254	1.257	1.253	1.254666667	0.002081666	28.53299706
pH 8	TW15	1.320	1.322	1.320	1.320666667	0.001154701	50.6897061
	TW17	1.319	1.321	1.320	1.32	0.001	50.46590096
	TW15+TW17	1.306	1.308	1.305	1.306333333	0.001527525	45.87789555
pH 9	TW15	1.386	1.389	1.388	1.387666667	0.001527525	73.18212285
	TW17	1.399	1.395	1.396	1.396666667	0.002081666	76.20349227
	TW15+TW17	1.371	1.374	1.375	1.373333333	0.002081666	68.3703123
pH 10	TW15	1.421	1.417	1.416	1.418	0.002645751	83.36525681
	TW17	1.429	1.433	1.432	1.431333333	0.002081666	87.84135964
	TW15+TW17	1.411	1.408	1.409	1.409333333	0.001527525	80.45578996

3.3 Optimization of Temperature

There was a significant degradation of chromium by TW15(Paracoccus pantotrophus), TW17(Bacillus velezensis) and TW15(Paracoccus pantotrophus)+TW17(Bacillus velezensis) seen at temperature of 30°C and 35°C, in which maximum degradation took place at 35°C

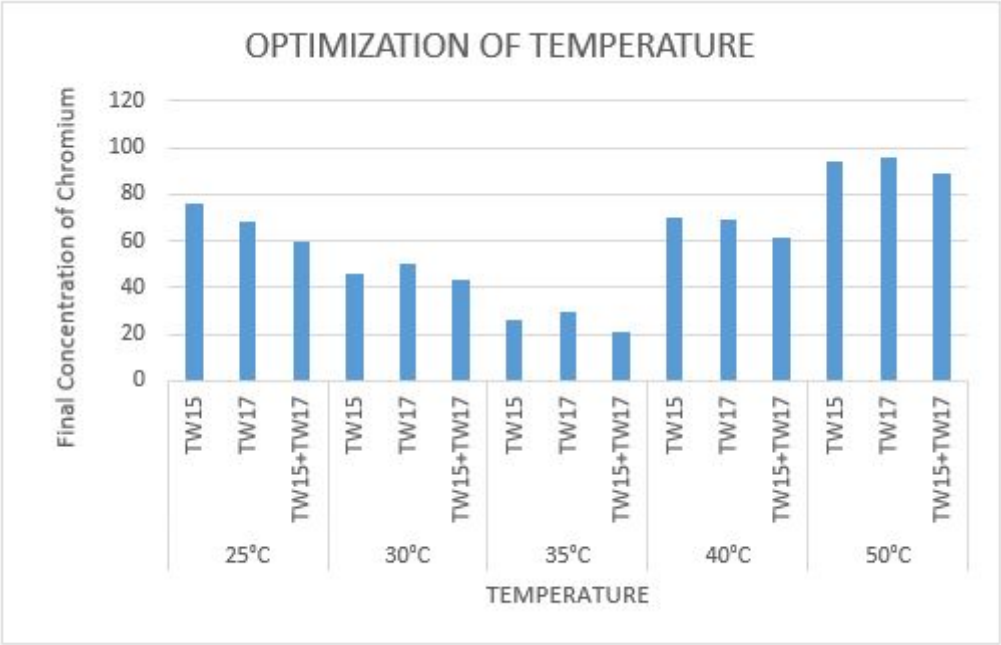

FIGURE 4

Optimization of Temperature

3.4 Optimization of Carbon Sources:

Chromium degradation by TW15(Paracoccus pantotrophus) was higher in the media supplemented with Fructose as the carbon source, whereas TW17(Bacillus velezensis) showed a maximum chromium degradation in media that contained Glucose as the carbon source. Thus, TW15(Paracoccus pantotrophus)+TW17(Bacillus velezensis) together showed a significant amount of chromium degradation in media that used Glucose and Fructose as carbon source.

3.5 Optimization of Nitrogen Sources:

A significant amount of chromium was degraded by TW15(Paracoccus pantotrophus) in the media containing Yeast Extract as the nitrogen source, whereas degradation by TW17(Bacillus velezensis) was higher in the media with Peptone and TW15(Paracoccus

Table 4

Optimization of Temperature

TEMPERATURE	ORGANISM	OD1	OD2	OD3	MEAN	STD DEV	Final Concentration of Chromium
25°C	TW15	1.395	1.397	1.396	1.396	0.001	75.97968713
	TW17	1.373	1.372	1.374	1.373	0.001	68.25840973
	TW15+TW17	1.348	1.345	1.347	1.346666667	0.001527525	59.41810663
30°C	TW15	1.308	1.309	1.301	1.306	0.004358899	45.76599298
	TW17	1.319	1.318	1.320	1.319	0.001	50.13019324
	TW15+TW17	1.299	1.299	1.302	1.3	0.001732051	43.7517467
35°C	TW15	1.246	1.247	1.248	1.247	0.001	25.95923793
	TW17	1.259	1.257	1.257	1.257666667	0.001154701	29.5401202
	TW15+TW17	1.233	1.231	1.234	1.232666667	0.001527525	21.14742738
40°C	TW15	1.379	1.377	1.376	1.377333333	0.001527525	69.71314316
	TW17	1.376	1.378	1.373	1.375666667	0.002516611	69.1536303
	TW15+TW17	1.351	1.355	1.352	1.352666667	0.002081666	61.43235291
50°C	TW15	1.448	1.450	1.451	1.449666667	0.001527525	93.99600105
	TW17	1.455	1.453	1.454	1.454	0.001	95.45073447
	TW15+TW17	1.436	1.433	1.434	1.434333333	0.001527525	88.84848278

Table 5

Optimization of carbon sources

CARBON SOURCES	ORGANISM	OD1	D2	OD3	MEAN	STD DEV	FINAL CONC. OF CHROMIUM
GLUCOSE	TW15	1.332	1.333	1.328	1.331	0.002645751	54.1586858
	TW17	1.285	1.285	1.287	1.285666667	0.001154701	38.93993615
	TW15+TW17	1.265	1.263	1.247	1.258333333	0.009865766	29.76392534
STARCH	TW15	1.327	1.330	1.329	1.328666667	0.001527525	53.3753678
	TW17	1.354	1.353	1.352	1.353	0.001	61.54425548
	TW15+TW17	1.312	1.314	1.314	1.313333333	0.001154701	48.22784954
FRUCTOSE	TW15	1.273	1.276	1.275	1.274666667	0.001527525	35.24715131
	TW17	1.341	1.340	1.342	1.341	0.001	57.51576293
	TW15+TW17	1.252	1.250	1.249	1.250333333	0.001527525	27.07826364
LACTOSE	TW15	1.332	1.336	1.328	1.332	0.004	54.49439351
	TW17	1.338	1.336	1.340	1.338	0.002	56.50863979
	TW15+TW17	1.325	1.324	1.321	1.323333333	0.002081666	51.58492667
SUCROSE	TW15	1.333	1.336	1.334	1.334333333	0.001527525	55.27771151
	TW17	1.343	1.340	1.341	1.341333333	0.001527525	57.6276655
	TW15+TW17	1.314	1.317	1.315	1.315333333	0.001527525	48.89926496

pantotrophus)+TW17(Bacillus velezensis) showed a maximum degradation in the media with Glucose and Peptone as the Nitrogen source

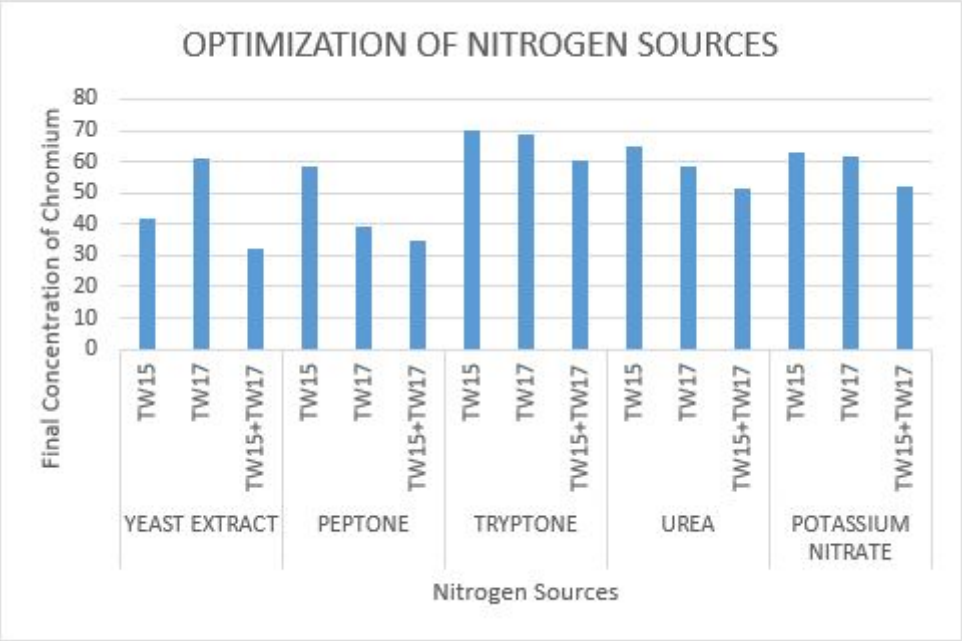

FIGURE 5

Optimization of Nitrogen Source

3.6 Optimization of Inoculum Concentration

More the concentration of the inoculum added to the media, the amount of chromium degradation seemed to have a gradual increase

3.7 Optimization of Chromium Concentration :

The value of chromium degradation by TWI5(Paracoccus pantotrophus), TW17 and TW15(Paracoccus pantotrophus)+TW17(Bacillus velezensis) seems show a trend of gradual decrease with the increase in the concentration of chromium added to the media.[1]

[1]

Table 6

Optimization of Nitrogen Source

NITROGEN SOURCES	ORGANISM	OD1	OD2	OD3	MEAN	STD DEV	FINAL CONC.OF CHROMIUM
YEAST EXTRACT	TW15	1.297	1.293	1.294	1.294666667	0.002081666	41.96130557
	TW17	1.353	1.354	1.35	1.352333333	0.002081666	61.32045034
	TW15+TW17	1.265	1.268	1.263	1.265333333	0.002516611	32.11387933
PEPTONE	TW15	1.327	1.328	1.374	1.343	0.026851443	58.18717835
	TW17	1.285	1.288	1.284	1.285666667	0.002081666	38.93993615
	TW15+TW17	1.272	1.274	1.271	1.272333333	0.001527525	34.46383332
TRYPTONE	TW15	1.381	1.379	1.377	1.379	0.002	70.27265601
	TW17	1.375	1.373	1.375	1.374333333	0.001154701	68.70602002
	TW15+TW17	1.348	1.349	1.352	1.349666667	0.002081666	60.42522977
UREA	TW15	1.364	1.363	1.361	1.362666667	0.001527525	64.78943004
	TW17	1.343	1.345	1.341	1.343	0.002	58.18717835
	TW15+TW17	1.324	1.325	1.322	1.323666667	0.001527525	51.69682924
POTASSIUM NITRATE	TW15	1.357	1.358	1.357	1.357333333	0.00057735	62.9989889
	TW17	1.353	1.356	1.354	1.354333333	0.001527525	61.99186576
	TW15+TW17	1.325	1.325	1.327	1.325666667	0.001154701	52.36824466

Table 7

Optimization of Inoculum Concentration

INOCULUM CONC.	ORGANISM	OD1	OD2	OD3	MEAN	STD DEV	FINAL CONC. OF CHROMIUM
0.50%	TW15	1.459	1.457	1.456	1.457333333	0.001527525	96.56976018
	TW17	1.462	1.46	1.463	1.461666667	0.001527525	98.0244936
	TW15+TW17	1.450	1.449	1.452	1.450333333	0.001527525	94.21980619
1.00%	TW15	1.426	1.428	1.429	1.427666667	0.001527525	86.61043136
	TW17	1.422	1.42	1.423	1.421666667	0.001527525	84.59618509
	TW15+TW17	1.413	1.411	1.414	1.412666667	0.001527525	81.57481567
2.00%	TW15	1.403	1.401	1.405	1.403	0.002	78.32964112
	TW17	1.409	1.411	1.408	1.409333333	0.001527525	80.45578996
	TW15+TW17	1.392	1.396	1.395	1.394333333	0.002081666	75.42017427
3.00%	TW15	1.345	1.346	1.348	1.346333333	0.001527525	59.30620406
	TW17	1.352	1.350	1.353	1.351666667	0.001527525	61.0966452
	TW15+TW17	1.324	1.321	1.322	1.322333333	0.001527525	51.24921895
4.00%	TW15	1.287	1.286	1.285	1.286	0.001	39.05183872
	TW17	1.273	1.272	1.270	1.271666667	0.001527525	34.24002817
	TW15+TW17	1.250	1.248	1.247	1.248333333	0.001527525	26.40684821
5.00%	TW15	1.237	1.235	1.236	1.236	0.001	22.26645309
	TW17	1.233	1.230	1.231	1.231333333	0.001527525	20.69981709
	TW15+TW17	1.220	1.221	1.220	1.220333333	0.00057735	17.00703225

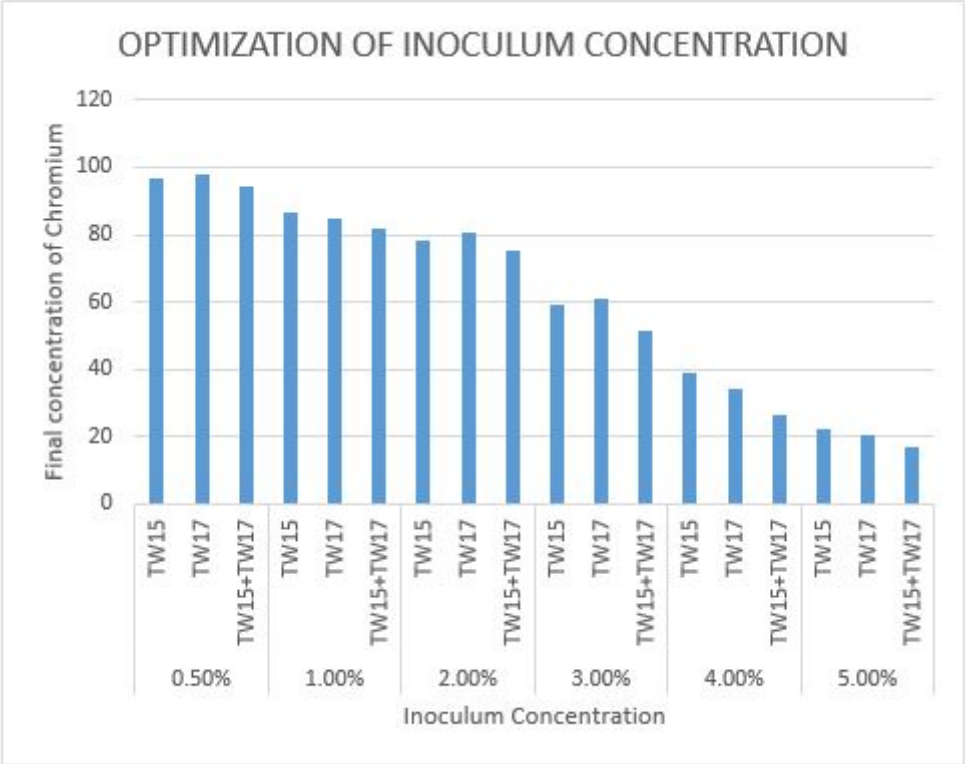

FIGURE 6

Optimization of inoculum concentration

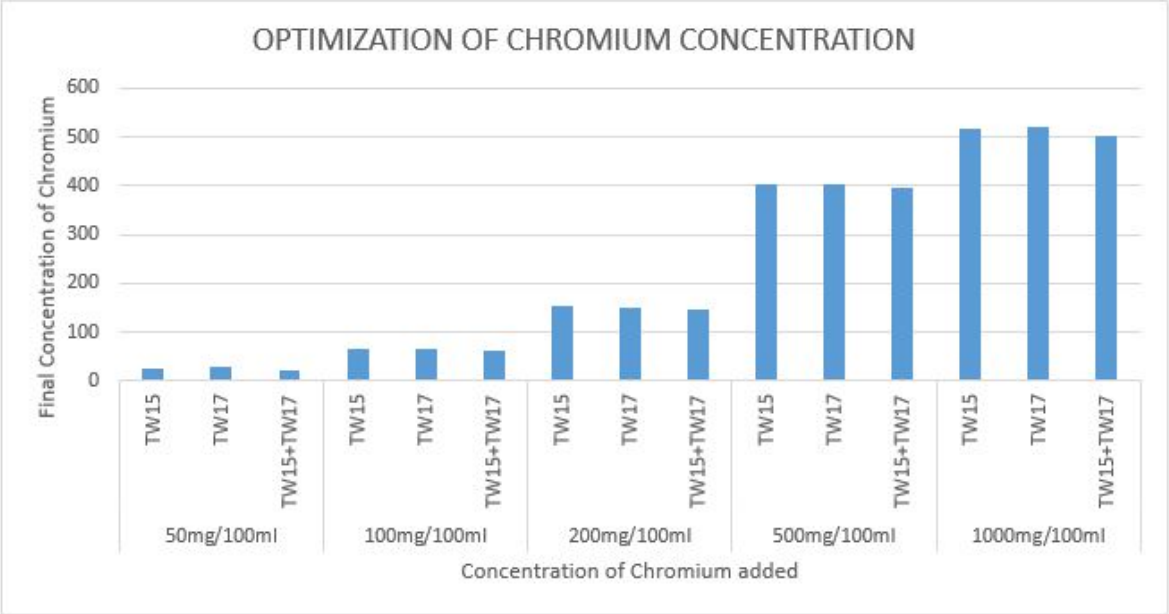

FIGURE 7

Optimization of Chromium concentration

Table 8

Optimization of chromium concentration

CHROMIUM CONC.	ORGANISM	OD1	OD2	OD3	MEAN	STD DEV	FINAL CONC. OF CHROMIUM
50mg/100ml	TW15	1.249	1.244	1.245	1.246	0.002645751	25.62353021
	TW17	1.257	1.258	1.260	1.258333333	0.001527525	29.76392534
	TW15+TW17	1.236	1.233	1.236	1.235	0.001732051	21.93074537
100mg/100ml	TW15	1.363	1.364	1.362	1.363	0.001	64.90133261
	TW17	1.365	1.362	1.364	1.363666667	0.001527525	65.12513775
	TW15+TW17	1.353	1.354	1.351	1.352666667	0.001527525	61.43235291
200mg/100ml	TW15	1.626	1.624	1.624	1.624666667	0.001154701	152.7448508
	TW17	1.618	1.621	1.619	1.619333333	0.001527525	150.9544096
	TW15+TW17	1.609	1.611	1.607	1.609	0.002	147.4854299
500mg/100ml	TW15	2.370	2.372	2.373	2.371666667	0.001527525	403.5185122
	TW17	2.365	2.368	2.367	2.366666667	0.001527525	401.8399736
	TW15+TW17	2.348	2.344	2.345	2.345666667	0.002081666	394.7901117
1000mg/100ml	TW15	2.713	2.719	2.714	2.715333333	0.00321455	518.8900628
	TW17	2.724	2.717	2.721	2.720666667	0.003511885	520.680504
	TW15+TW17	2.672	2.673	2.660	2.668333333	0.007234178	503.1118003

3.8 Optimization of Incubation Period

Maximum chromium degradation observed after the incubation from Day 7 to Day 28, by TW15 (Paracoccus pantotrophus), TW17 (Bacillus velezensis) and TW15 (Paracoccus pantotrophus)+TW17 (Bacillus velezensis).

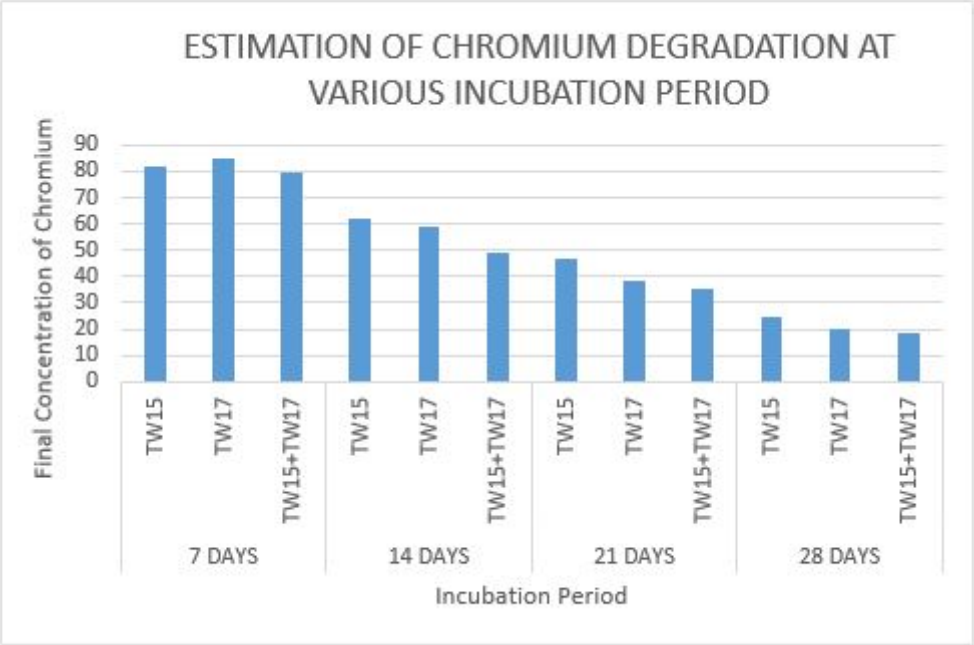

FIGURE 8

Optimization of incubation period

TABLE 9

Optimization of incubation period

INCUBATION PERIOD	ORGANISM	OD1	OD2	OD3	MEAN	STD DEV	FINAL CONC. OF CHROMIUM
7 DAYS	TW15	1.414	1.414	1.413	1.413666667	0.00057735	81.91052339
	TW17	1.422	1.425	1.423	1.423333333	0.001527525	85.15569794
	TW15+TW17	1.408	1.405	1.409	1.407333333	0.002081666	79.78437454
14 DAYS	TW15	1.353	1.355	1.357	1.355	0.002	62.2156709
	TW17	1.348	1.347	1.344	1.346333333	0.002081666	59.30620406
	TW15+TW17	1.314	1.318	1.315	1.315666667	0.002081666	49.01116754
21 DAYS	TW15	1.305	1.309	1.310	1.308	0.002645751	46.4374084
	TW17	1.285	1.283	1.284	1.284	0.001	38.3804233
	TW15+TW17	1.272	1.275	1.275	1.274	0.001732051	35.02334617
28 DAYS	TW15	1.245	1.244	1.241	1.243333333	0.002081666	24.72830965
	TW17	1.231	1.229	1.227	1.229	0.002	19.9164991
	TW15+TW17	1.225	1.225	1.223	1.224333333	0.001154701	18.3498631

4 Conclusion

In the present study potent indigenous bacterial species are Paracoccus pantotrophus and Bacillus velezensis individualy and mixed cultures were used to determine the biodegradation of chromium in optimized conditions. This study reveals the most significant parameters which contribute to the maximum chromium removal efficiency of the test micro organism at the optimal conditions.. Therefore, water to be treated biologically before discharge into water bodies.

5 Acknowledgment

Authors thank the Director ,Sangene Biotech,Bengaluru for providing necessary the facilities to conduct the research work.

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