

CHARACTERIZATION AND TREATMENT OF INDUSTRIAL EFFLUENTS USING ADAPTED CONSORTIUM IN FIXED FILM BIOREACTOR

V.Y. Shukla^{1*}, H. G. Pansuriya²¹Assistant Professor, ²Junior Research Fellow, Shree P.M. Patel Institute of P.G. Studies and Research in Science, Sardar Patel Uni., Gujarat, India.***Corresponding Author:**

E-mail: viral3007@gmail.com

ABSTRACT

Background: Wastewater from various industrial plants such as paper and pulp mills, pigment and textiles can contain various substances that tend to increase chemical oxygen demand (COD) of wastewater. Various agencies like Central Pollution Control Board (CPCB) have placed limits on allowable levels of COD in industrial wastewater effluents. It is desirable to develop a process suitable for treating wastewater to meet the regulatory limits.

Objective: The present study was attempted to solve the problem of high COD values as well as concerned heavy metals like Cu^{+2} and Cr^{+6} in industrial wastewater using native bacteria.

Methods: The organic matter that contributed to high COD in wastewater was degraded by native bacterial consortium under aerobic conditions using batch process. Flask treatment experiment was carried out to optimize inoculum size, temperature, pH and HRT using consortium for effective COD, Cu^{+2} and Cr^{+6} removals. Lab scale reactor study was carried out in which bacteria were allowed to attach to a supporting matrix to prevent slough off during treatment.

Results: Under the experimental conditions, the reduction efficiencies of COD, Cu^{+2} and Cr^{+6} were found to be as high as 57%, 97% and 93% respectively with 72h HRT in fixed film bioreactor.

Conclusion: Use of native biological population for the treatment of industrial wastewater is a permissible and cost effective technique.

Keywords: Wastewater treatment, COD, Heavy metals, Bio removal, Fixed film bioreactor

INTRODUCTION

Since the turn of the century, biologists have struggled to determine the impact of anthropological activity on aquatic biota. Wastewater also contains nutrients, which can stimulate the growth of aquatic plants. It may contain toxic compounds that are potential mutagen or carcinogen. For these reasons, the immediate and nuisance-free removal of wastewater from its source of generation, followed by treatment, reuse, or dispersal into the environment is necessary to protect public health and the environment^[1].

In 1860, for the first time, an attempt was made to control especially water and atmospheric pollution through criminal sanctions under the Indian Penal Code, 1860. The Water (Prevention and Control of Pollution) Act, 1974, provides for the establishment of pollution control boards at Centre and States to keep a watch over prevention and control of pollution. Biological treatment of wastewater is evaluated as a good method for industrial effluents. Bacterial treatment of wastes involves the stabilization of waste by decomposing them into harmless inorganic solids by aerobic or anaerobic process. The selection of bacterial species for the biological treatment depends upon the chemical composition of the effluent^[2]. *Bacillus subtilis* and other species of genus *Bacillus* form bio films at the air-liquid interface which is called pellicle. So the bacterial treatment using these

organisms is a permissive perspective^[3]. Various aerobic bacteria like *Pseudomonas fluorescens*, *Bacillus fusiformis*, *Staphylococcus aureus*, *Aeromonashydrophila*, *Brevibacilluslatrospor*, *Enterococcus faecalis* etc. are capable of dye decolorization^[4]. Bio augmentation (the process of adding selected strains/mixed cultures to wastewater reactors to improve the catabolism of specific compounds, (e.g. refractory organics, or overall COD) is a promising technique to solve practical problems in wastewater treatment plants, and enhance removal efficiency^[5]. The approach of bio augmentation focuses on taking advantage of microbial consortia designed for the specific physico-chemical properties of the bioprocess, since this approach was shown to be more efficient than using undefined inocula^[6]. Potentially higher efficiencies can be achieved in systems such as membrane bioreactor which can immobilize bacteria and prevent slough-off where the environmental conditions can be manipulated to enhance survival and prolong the activity of the exogenous population^[7]. Advanced oxidation technologies like Fenton's process gives COD removal up to 70% but has been proven costly and polluting itself^[8].

One of the most important chemical contaminants of concern is Chromium (Cr), which exists in a series of oxidation states from -2 to +6 valence; the most important stable states are 0 (element metal), +3 (trivalent) and +6 (hexavalent).

Cr^{+3} and Cr^{+6} are released to the environment primarily from stationary point sources resulting from human activities. Cr in such solutions mostly occurs as oxyacids and oxyanions of Cr^{+6} [9].

Copper is an essential element for all living organisms^{[10];[11]} but at higher concentrations, it inhibits cell metabolism. Cu and Cu-containing compounds are widely used as bactericides and fungicides. The antimicrobial action of Cu is believed to result from the ability of Cu^{+2} to chelate sulfhydryl groups, thereby interfering with the cell proteins or enzymes^[12]. The results of a study demonstrated that soluble Cu can potentially be a severe inhibitor of various microbial populations involved in the removal of organic matter (i.e., fermentative bacteria, aerobic heterotrophs) and nitrogen nutrient removal (i.e., nitrifying and denitrifying bacteria) during biological waste water treatment^[13].

MATERIALS AND METHODS

Seven different samples were collected from different sites/industries of Ankleshwar and Vapi industrial area in Gujarat, India in sterile plastic containers with minimum amount of 1L each. Samples were preserved at 4°C up till the analysis.

Table 1: Sources of collected samples

Sample no.	Description (Source)
1)	Dyeing industry, Vapi GIDC
2)	Vapi khadi (Effluent Sewage)
3)	Pigment industry, Ankleshwar GIDC
4)	Pigment intermediate industry, Ankleshwar GIDC
5)	Healthcare product industry, Ankleshwar GIDC
6)	Paper and pulp mill, Ankleshwar GIDC
7)	Textile production industry, Panoli GIDC

Characterization of wastewater samples:

Physiological characterization of all samples was done in terms of acidity, alkalinity, TS, TDS and TSS. pH was measured using pH meter. Chemical analysis of wastewater was done in terms of COD and BOD. The samples were also analyzed for copper and chromium using Atomic absorption spectrometer^[14], the ContrAA 300 (Analytik Jena) air-acetylene flame spectrometer (Photograph 1) equipped with xenon short-arc lamp as a continuum radiation source for all elements, double monochromator consisting of a prism pre-monochromator and an echelle grating monochromator, and CCD (charge coupled device) as detector used. Cu^{+2} estimation at **324.8 nm** and Chromium estimation at **357.9nm** were carried out with the help of Aspect CS Software with Windows XP compatibility.



Photograph 1: Analytic Jena, Germany [contrAA300] AAS

Isolation and screening of microorganisms:

Bacterial diversity present in the samples was studied on HPC agar. Appropriate dilutions (ranging from 10^{-1} to 10^{-12}) of the samples were made and plated on the medium to obtain isolation of different native bacteria. All the plates were incubated at $32 \pm 2^\circ\text{C}$ for 48h and CFU counting from all the plates was done. Isolation was done on nutrient agar medium and preserved on nutrient agar slants. Colony characters were determined in terms of colony morphology like size, shape, surface, edge, elevation, transparency, pigmentation and consistency. Morphological characters of microorganisms were studied in terms of Gram reaction, shape and cellular arrangements^[14].

From isolated cultures, most common and fast growing isolates were selected for further studies. A consortium was prepared by taking equal amount of seven selected overnight grown liquid cultures which were set equivalent to 0.5 McFarland standards^[14].

Effect of different parameters

Effect of inoculum size on growth pattern of consortium:

Prepared consortium was screened for its % culture inoculation for different time interval. Prepared consortium was added to respective flasks containing sterile nutrient broth in two different quantities which represented 5% and 10% culture system. Both the flasks were incubated in a shaker incubator [NOVA] at 37°C with 150rpm. Samples were withdrawn at different time intervals and growth density was recorded on UV-VIS spectrophotometer [SPECORD 205] at 600nm.

Effect of temperature and pH on growth pattern of consortium:

pH, temperature, ionic strength, biosorbent dosage, bio sorbent size, initial solute concentration, agitation rate are important factors in the evaluation of the full bio removal potential of any

biomaterial^[15]. So an attempt was made to optimize some of these factors for the proposed technique.

Seventeen sterile nutrient broth flasks except control (C) were prepared to be inoculated with 5% consortium suspension. Four sets of flasks with different pH (i.e. 5.0, 6.0, 7.0 and 8.0) were incubated at different temperatures like 27°C, 32°C, 37°C and 42°C. An agitation rate of 150rpm was constant for all shake-flask studies described here and further in this study. Samples were withdrawn aseptically from each flask at different time intervals and growth density was recorded on UV-VIS spectrophotometer at 600nm.

Flask treatment of original and simulated waste samples with selected consortium

COD removal from preliminary treated effluent sample: Effluent samples were pretreated by filtering through filter paper for grit removal as preliminary treatment.

Under aseptic condition, all the flasks except control (C) were inoculated with 5% consortium suspension (as per 3.2) and pretreated effluent sample was added to it. Four sets of flasks with different pH (i.e. 5.0, 6.0, 7.0 and 8.0) were incubated at different temperatures like 27°C, 32°C, 37°C and 42°C at 150rpm. Samples were withdrawn aseptically at different time intervals and COD levels were determined to observe flask-treatment efficacy.

Cu⁺² and Cr⁺⁶ reduction in simulated waste sample: Two sets of seventeen flasks containing nutrient broth in each were prepared as described in 3.3.2 for Cu⁺² and Cr⁺⁶ reduction assay. Four sets of nutrient broth flasks were adjusted at different pH viz. 5.0, 6.0, 7.0 and 8.0 with the help of 1N HCl or 1N NaOH. Standard solutions of Cu⁺² and Cr⁺⁶ (1000mg/L) were prepared from CuSO₄.5H₂O and K₂Cr₂O₇ respectively and sterilized separately. The control flasks contained 50mg/L of Cu⁺² and Cr⁺⁶ in each.

Under aseptic condition, solutions containing 50mg/L initial concentration of Cu⁺² and Cr⁺⁶ were added to respective flasks and all the flasks except control (C) were inoculated with 5% consortium suspension (as per 3.2). All the flasks were incubated as described in 3.4.1. Samples were withdrawn aseptically at different time intervals and metal ion estimation was carried out by Atomic Absorption Spectroscopy [contraA300] for detection of flask-treatment effectiveness.

Scale Up (Treatment of effluent samples in indigenously prepared bioreactor)

Micro aerophilic fixed film bioreactor: A bioreactor (Photograph 2) was made of sterile plastic container measuring 25cm(length) × 10cm(ID). Sterile glass resin beads measuring 7mm-12mm(length) × 10mm(ID) were used as supporting

matrix and were made rough by using emery paper (Table 2). A sample outlet was made at bottom end of the container to make it down flow. A study by Kim *et al.*, 2003^[16] suggests that support media is advantageous for achieving efficient treatment of wastewater. They also reported that using support media and selected microorganisms prevents generation of large amount of mixed liquor suspended solids.

Table 2: Configuration of indigenously prepared bioreactor

Details	Configuration
Vessel material	Polythene
Vessel dimension [Height:Diameter] (cm)	25:10:00
Actual working dimension	20:10
Capacity (ml)	1000
Working volume (ml)	750
Medium	Nutrient broth
Medium volume (ml)	750
Inoculums	Consortium of all seven isolates
Inoculums size (%v/v)	1.6×10 ⁸ cells/ml approx.
pH	5.2
Temperature (°C)	32±2



Photograph 2: Indigenously prepared fixed film bioreactor

Effluent treatment in microaerophilic bioreactor:

Consortium was prepared as described in 3.2 according to the fluid capacity of the container. Prepared consortium was poured in the bioreactor, so that the support matrix would completely submerge under it. Further, biofilm was allowed to develop over the matrix under aerobic conditions up to 3days. After satisfactory biofilm development, 200ml initial fluid was taken out from the outlet and 200ml of the preliminary treated mixed effluent of dyeing industry effluent + paper and pulp mill effluent (100mL + 100mL) was added to bioreactor. As the system was open, micro aerophilic conditions developed. Sample was withdrawn from the bottom at different time

intervals and analyzed for COD, Cu^{+2} and Cr^{+6} reductions.

RESULTS AND DISCUSSIONS

Characterization of wastewater samples:

Important physicochemical characters are shown in table 2. Sample no.1 collected from a dyeing industry at Vapi was having highest alkalinity. It was containing abnormal amounts of Copper and Chromium. Sample no.3 collected from a pigment industry at Ankleshwar was the most acidic with pH of 1.8, followed by Sample no.4 having pH of 2.7. Sample no.5 collected from a healthcare product industry was found to contain highest acidity. Sample no.6 collected from a paper and pulp mill at Ankleshwar was shown to contain abnormally high contents of TDS, TSS and TS. COD and BOD were also found to be present far above normal limits in it. Sample no.7; a textile industry wastewater, was found to be least toxic among all samples collected.

N.B.: N.A. indicates that sample is not suitable for that characteristic to be determined.

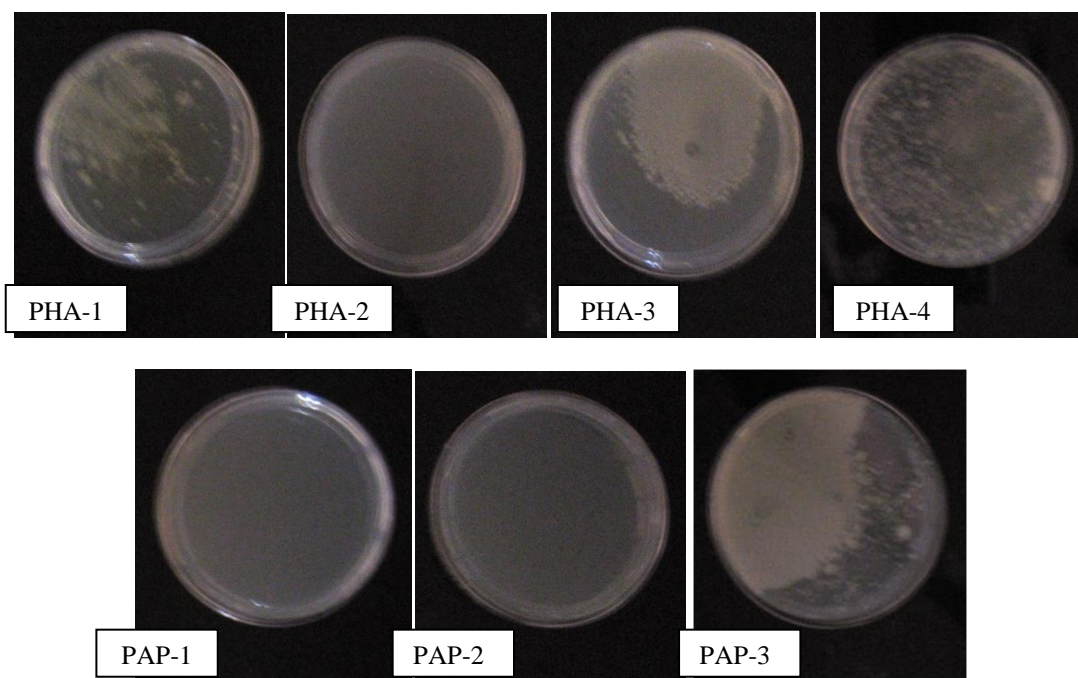
Isolation and screening of the microorganisms:

Isolation study showed that no bacterial population was present in the sample no.3 and 4 mainly because of much higher acidic pH. Maximum no. of microbial diversity was found to be present in the sample no.6 which was 4.2×10^{10} CFU/mL. Whereas sample no.1 was found to be having the lowest no. of microbial population which was 1.01×10^5 CFU/mL. Sample no.2, 5 and 7 was having CFU count around 2.7×10^6 CFU/mL, 2.3×10^8 CFU/mL and 3.9×10^5 CFU/mL, respectively. All the samples showed the presence of Gram positive bacilli except one isolate which was found to be present in sample no.7. Sample no.5 and 6 showed maximum microbial diversity.

Colony characters are depicted in table no.2 and 3 for the selected isolates for further study (Photograph 3).

Table 3: Physicochemical characteristics of different wastewater samples

Sample no. →	1	2	3	4	5	6	7	CPCB ^[17]
Characteristics ↓								
Color	Dark brown	Colorless	Pale yellow	Light blue	Slight green	Brown	Colorless	N.A.
Turbidity	Slight turbid	Clear	Clear	Slight turbid	Clear	Turbid	Slight turbid	N.A.
pH	4.5	5.5	1.8	2.7	4.6	4.4	6.8	5.5-9.0
Acidity (mg/L)	625	62.5	N.A.	N.A.	2350	285	125	N.A.
Alkalinity (mg/L)	600	200	N.A.	N.A.	250	65	130	N.A.
TDS (mg/L)	8545	6000	N.A.	N.A.	5650	16435	565	N.A.
TSS (mg/L)	2010	1025	N.A.	N.A.	145	38340	1215	100
TS (mg/L)	10555	7025	N.A.	N.A.	5795	54775	1780	N.A.
COD (mg/L)	2600	146	4782	1100	8936	12249	546	250
BOD ₅ (mg/L)	200	16	N.A.	N.A.	260	490	88	30
Cu^{+2} (mg/L)	28	26	0	0	14.4	27.05	0.26	3
Cr^{+6} (mg/L)	250.7	3.5	0	0	0.47	2.67	0.024	0.1



Photograph 3: Selected isolates

Table 4: Colony characters of isolates (1)

Character	PHA-1	PHA-2	PHA-3	PHA-4
Size	Large	Medium	Small	Small
Shape	Round	Round	Round	Round
Surface	Rough	Smooth	Smooth	Smooth
Edge	Undulate	Entire	Entire	Entire
Elevation	Slightly Raised	Slightly Raised	Slightly Raised	Convex
Transparency	Opaque	Translucent	Opaque	Opaque
Pigmentation	No pigmentation	No pigmentation	No pigmentation	Pale yellow
Consistency	Dry	Moist	Mucoid	Moist
Gram staining	Gram positive bacilli with spores	Gram positive bacilli in single or double	Gram positive thick bacilli	Gram negative short rods
Motility	Motile	Motile	Motile	Motile

Table 5: Colony characters of isolates (2)

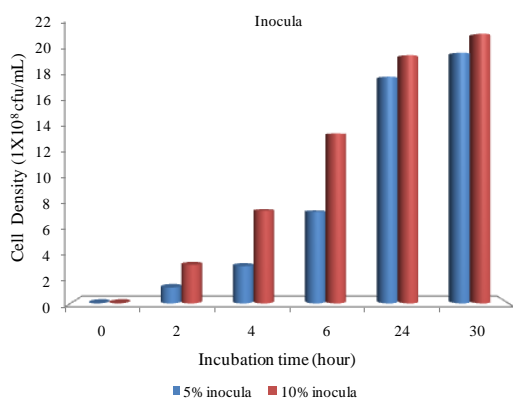
Character	PAP-1	PAP -2	PAP -3
Size	Medium	Large	Large
Shape	Round	Round	Round
Surface	Smooth	Rough	Smooth
Edge	Entire	Entire	Entire
Elevation	Slightly Raised	Slightly Raised	Slightly Raised
Transparency	Translucent	Opaque	Opaque
Pigmentation	No pigmentation	No pigmentation	No pigmentation
Consistency	Moist	Dry	Mucoid
Gram staining	Gram variable bacilli in single or double	Gram variable bacilli with spores	Gram variable bacilli with spores
Motility	Motile	Motile	Motile

On the basis of biochemical tests (data not shown), selected isolates were tentatively identified as *Bacillus* sp., except one that was PHA-4 isolate which was identified as *Enterobacteriaceae* sp.

Effect of different parameters

Effect of inoculum size on growth pattern of consortium: Shake flask optimization of the consortium was done with 5% and 10% culture size. As shown in Graph no.1, there was no significant difference in cell density after 30h incubation time so 5% culture size was used for further study. Thus, smaller size of inoculum was enough for the effective treatment.

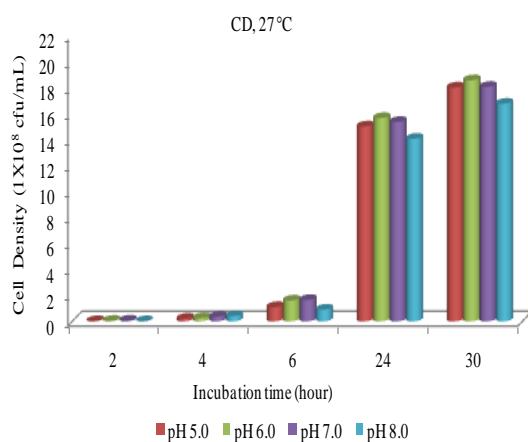
Graph 1: Influence of inoculum size on growth of consortium



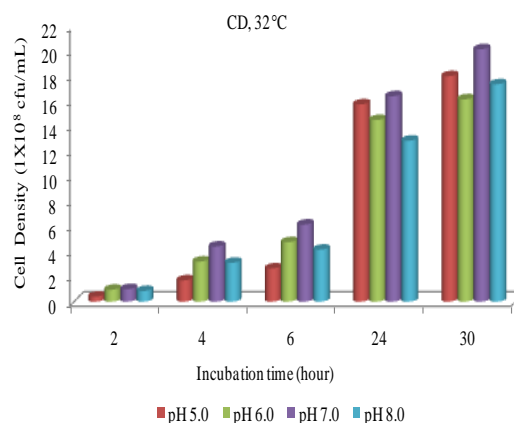
Effect of temperature and pH on growth pattern of consortium

Graph no.2-5 show the effect of change in temperature and pH on the growth of consortium at different time intervals. The data shows that the optimum temperature and pH for the growth of the consortium was 37°C and 7.0, respectively.

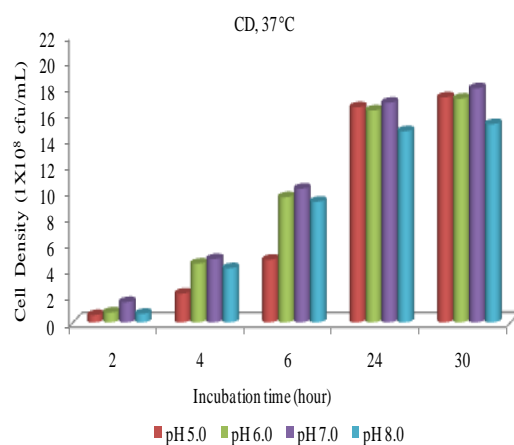
Graph 2: Influence of pH on growth of consortium at 27°C



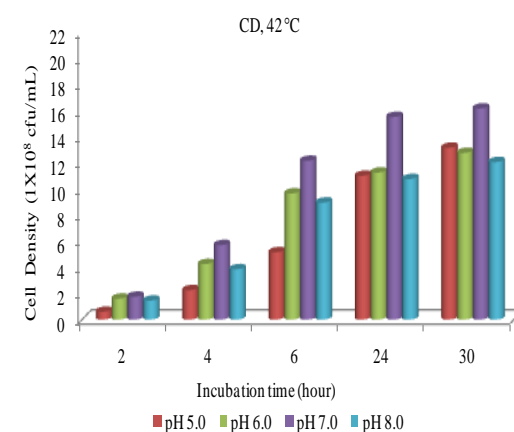
Graph 3: Influence of pH on growth of consortium at 32°C



Graph 4: Influence of pH on growth of consortium at 37°C



Graph 5: Influence of pH on growth of consortium at 42°C



Flask treatment of original and simulated waste samples with selected consortium

The percentage removal (η) calculated using the equation:

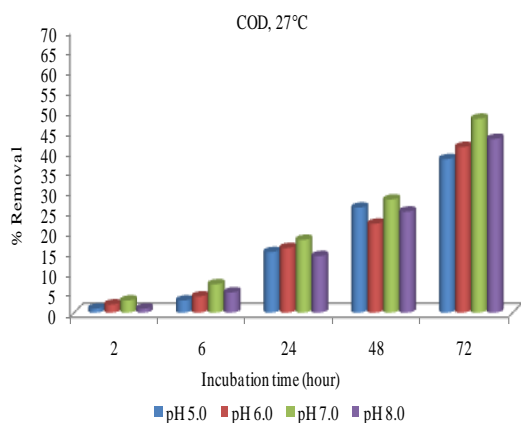
$$\eta(\%) = \frac{C1 - C2}{C1} \times 100$$

Where, C1 = Influent concentration and C2 = Effluent concentration^[18]

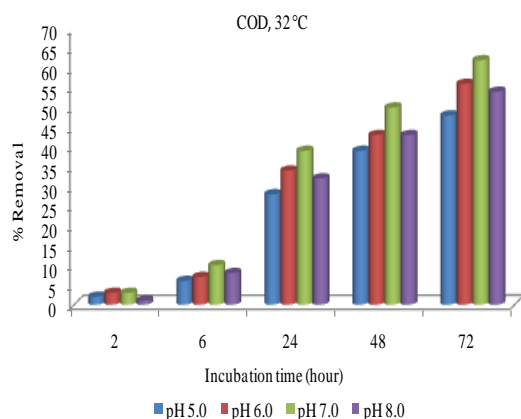
COD removal from preliminary treated effluent sample:

The data of COD removal from actual waste depicted in Graph no.6-9 indicates that at 27°C temperature and 7.0pH as high as 43% COD reduction could be achieved. At 32°C, COD reduction was increased up to 54% at pH 7.0 within 72h of incubation. Similar findings were observed at 37°C and 42°C also, which yielded 60% and 56% COD reduction respectively after 72h incubation. The data show that the optimum condition for the COD removal is 32°C-42°C and pH 7.0.

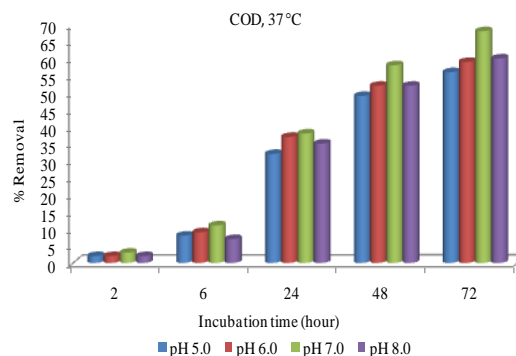
Graph 6: Influence of pH on sCOD removal at 27°C



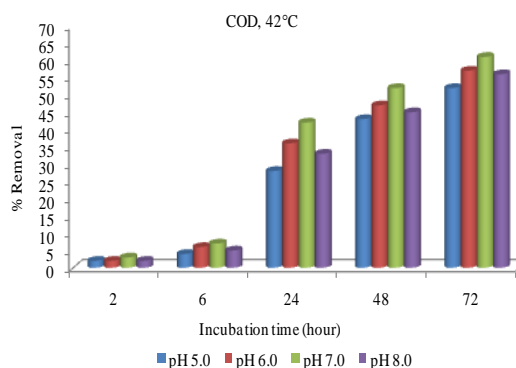
Graph 7: Influence of pH on sCOD removal at 32°C



Graph 8: Influence of pH on sCOD removal at 37°C



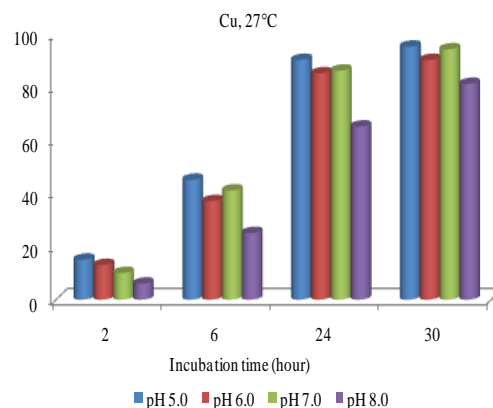
Graph 9: Influence of pH on sCOD removal at 42°C



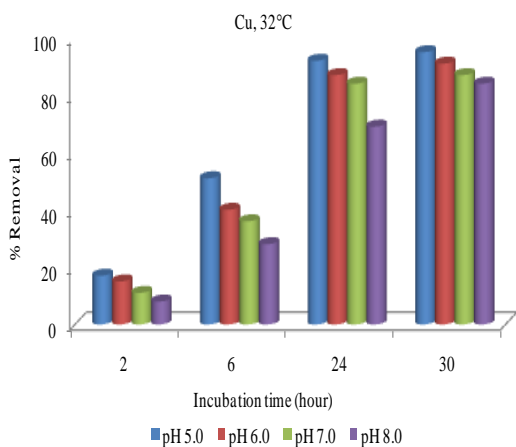
Cu⁺² and Cr⁺⁶ reductions in simulated waste sample

The results of copper removal from simulated waste are shown in Graph no.10-13 the data shows that 5.0 pH was optimum. The graph also indicates that as temperature increases, Cu-removal efficiency also increases; 95% removal at 27°C, 95% removal at 32°C, 97% removal at 37°C and 99% removal at 42°C within 30h of incubation time.

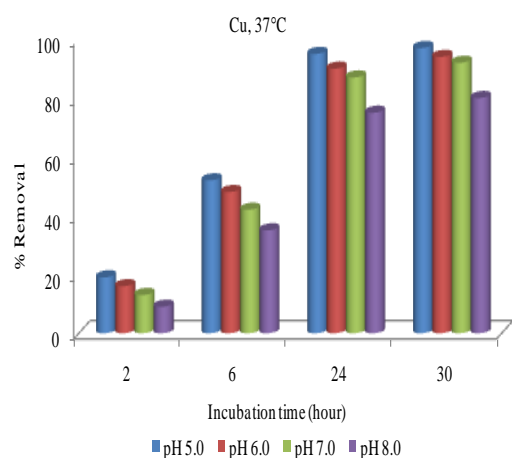
Graph 10: Influence of pH on Cu removal at 27°C



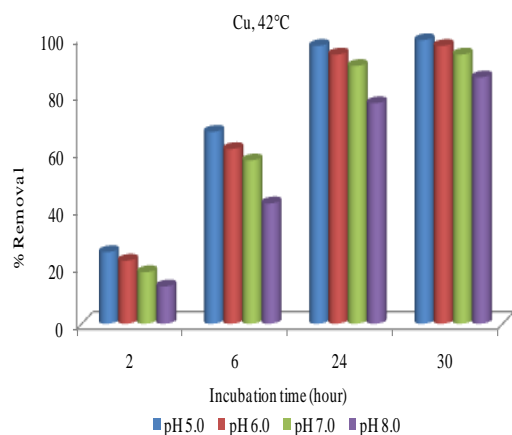
Graph 11: Influence of pH on Cu removal at 32°C



Graph 12: Influence of pH on Cu removal at 37°C

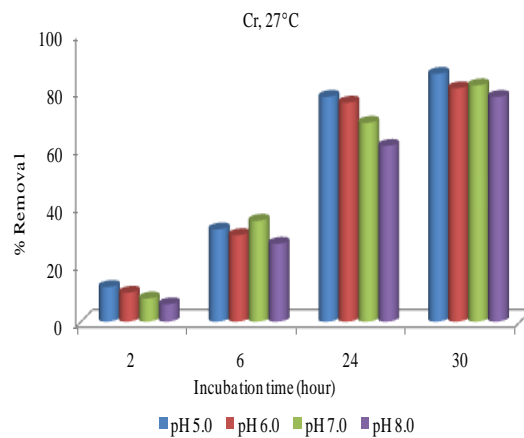


Graph 13: Influence of pH on Cu removal at 42°C

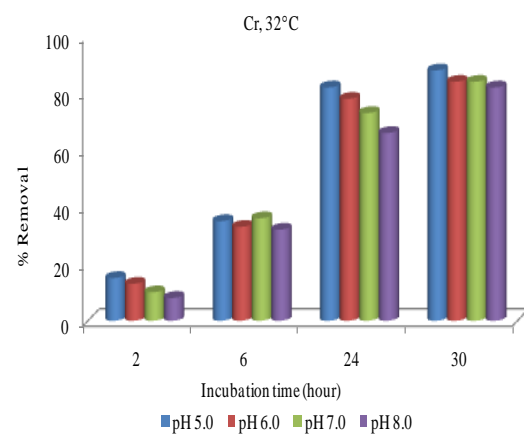


treatment by bacteria and their mixture system exhibited a successful power to treat wastewater. The study resulted in Cu⁺² and Cr⁺⁶ removal up to 63% and 67% respectively using *R. radiobacter*^[19].

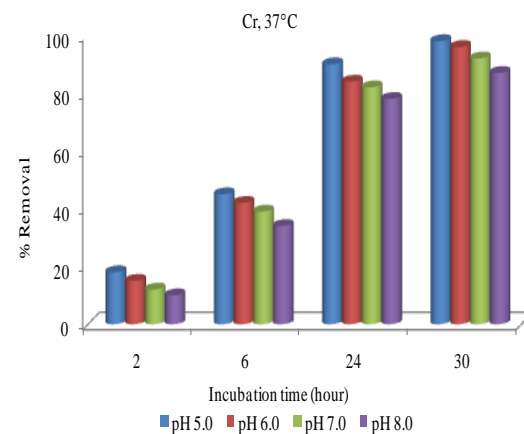
Graph 14: Influence of pH on Cr removal at 27°C



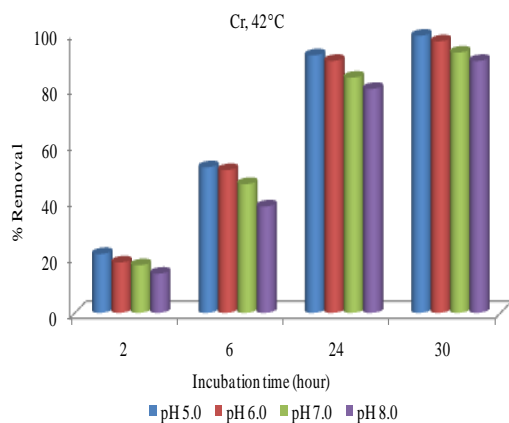
Graph 15: Influence of pH on Cr removal at 32°C



Graph 16: Influence of pH on Cr removal at 37°C

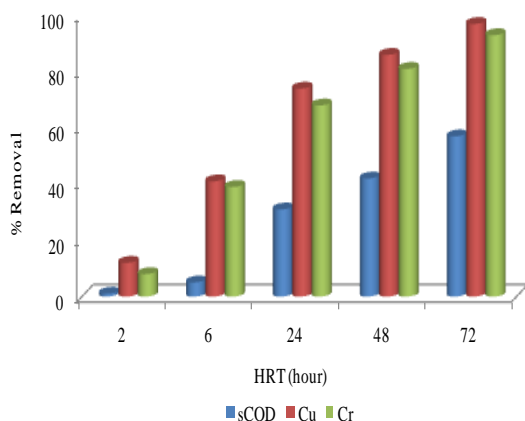


The chromium removal results shown in Graph no.14 to 17 indicate that the optimum pH for chromium removal was 5.0 whereas at 37°C, 98% removal was found after 30h of incubation. A study on sewage treatment from wastewater treatment plants in Egypt suggested that the biological

Graph 17: Influence of pH on Cr removal at 42°C**Scale Up (Treatment of effluent samples in indigenously prepared bioreactor):**

A lab scale reactor with 750ml hydraulic capacity was indigenously designed to carry out removal of heavy metals and COD from actual waste. The experiment was carried out in the reactor with 5% (v/v) inoculum of consortium and with actual waste prepared as described in 3.5 containing initial COD of 3675mg/L as soluble COD (sCOD) with pH 4.4.

The profile of copper, chromium and sCOD removal process in bioreactor using consortium for the period of 72h is shown in Graph 18. The results highlight the influence of hydraulic retention time for actual waste. Under the experimental conditions, COD reduction efficiency was found to be as high as 57%, copper reduction was 97% and chromium reduction was 93% within 72h of HRT. The used consortium showed better performance in static condition which provides micro aerophilic condition as compared to aerobic reactor.

Graph 18: Reactor study for 72h HRT

When compared with chemical methods such as Fenton-like process, which is operated at lower pH up to 2.5 also produces pollution itself,

bacteriological treatment does not need such pretreatment and has been proven harmless.

Biological technique proposed by Babuet *et al.*, 2000^[2] achieved 62% of COD removal from reactive dyeing effluent of a cotton textile industry. 42.4% and 63.6% COD reduction was reported by Kim *et al.*, 2003^[16] for bacteria without support media and bacteria with support media respectively. A similar study by Noorjahan CM, 2014^[20] from tannery effluent resulted in the isolation of bacteria like *Escherichia coli*, *Klebsiellasp.*, *Pseudomonas sp.* and *Staphylococcus aureus* and 91.6% COD removal was achieved using indigenous technique. A study has shown that *Phanerochaetechrysosporium* and *B. subtilis* rectified COD up to 94.4% and 94.7%, while *M. luteu* sand consortium decreased COD up to 89.6 and 92.7% respectively, under agitated conditions^[21]. A study was carried out by Raj *et al.*, 2014^[22] and *Paenibacillus sp.* strain LD1, a lac case producing bacterium was isolated from effluent contaminated site. The bacterium was used to treat pulp and paper mill effluent and removal efficiency of 78% COD were observed after 144 h of incubation.

Magnaye *et al.*, 2009^[23] carried out biodegradation studies using aerobic batch reactor and 98% reduction in COD was obtained in aerobic reactor with an HRT of 5 hours after 11 days at initial COD of 300ppm. A study on *Pleurotus spp.* in a lab scale RBC achieved 61.3% maximum COD reduction after 10days HRT^[24]. Kariminiaa-e-Hamedaaniet *al.*, 2003^[25] carried out biological treatment of wastewater from a food processing factory using packed bed reactor with ceramic carrier. They were able to achieve more than 87% COD reduction at 30.17h HRT with continuous aeration. An experiment carried out by Gupta *et al.*, 2001^[26] using *Aeromonasformicans* culture in a lab scale aerobic batch reactor resulted in 71% COD reduction after 10days HRT. Helble *et al.*, 1999^[27] used the combination of ozone with fixed bed biofilm reactors as a tertiary effluent treatment processes to give maximum COD reduction of 80%.

Chaturvedi, 2011^[28] isolated a potential bacterium from spent chrome effluent which was identified as *Bacillus circulans*MN1. The cells removed toxic Cr⁺⁶ more efficiently at 30°C temperature and optimum initial pH was 5.6. The maximum chromate removal (71.4%) at initial chromate concentration of 1110mg/L at 30°C was achieved during 24 hours of incubation period. Thus, the consortium was found to be a potent reducer of the Copper and Chromium from simulated waste. Abdullaet *al.*, 2010^[29] isolated chromate resistant actinomycetes and conducted combined chemical-biological study for tannery wastewater treatment in flask system. The technique achieved 99.5% Cr⁺⁶removal but COD reduction was only 36% after 96h HRT. A study carried out by using

Trichodermaviride obtained from chromium mud samples and 75mg/L Cr⁺⁶ was reduced completely after 14 days of incubation^[30].

A study was carried out by Majumder *et al*, 2015^[31] using an indigenous bacterial strain *Acinetobacter guillouiae* and mixture of compost and coal were used as a packing material for the bio filter column. The study resulted in a maximum removal (97.5%) of Cu⁺² during phase-II at lower inlet concentrations (17.5–20mgL⁻¹) and low flow rates (10mLmin⁻¹) of Cu⁺². Cu⁺² removal experiment using rice husk was carried out by Onget *et al*, 2003^[32] and stated that Cu removal increased from 24.49% to 98.177% with the increase of the amount of absorbent concentration, while Cu⁺² removal using fly ash varied from 37.38% to 98.545%. The copper-resistant strain KNP3 of *Proteus vulgaris* isolated from a power plant soil was used to treat soil and 55.6% Cu removal was observed^[33].

CONCLUSION

Sample no.2 collected from a pigment product industry at Ankleshwar was recorded with lowest pH. Sample no.5 collected from a health care product industry was found to contain highest acidity. Sample no.1 collected from a dyeing industry at Vapi was having highest alkalinity. Sample no.6 collected from a paper and pulp mill at Ankleshwar was shown to contain abnormally high contents of TDS, TSS and TS. COD and BOD were also found to be present far above normal limits in sample no.6. Sample no.1 contained abnormal amounts of Copper and Chromium. *Bacillus* sp. was found to be dominant in all the samples in terms of CFU/mL. Smaller size of inocula was enough for the effective treatment. Naturally occurring microorganisms in polluted water showed considerable copper and chromium removal ability. The optimum temperature and pH for the effective copper removal from the waste were 37°C and 5.0 respectively. Similarly, the optimum conditions for the effective chromium removal were 37°C temperature and pH 5.0. The optimum temperature for the maximum COD reduction was 37°C and pH 7.0. In a lab scale reactor study, COD reduction efficiency was found to be as high as 57%, Cu⁺² reduction was 97% and Cr⁺⁶ reduction was 93% within 72h of HRT. The removal process in indigenously designed bioreactor in the present investigation has distinct advantages viz.

1. No chemical additives or aeration requirements
2. No pH adjustment of the effluent was necessary as preliminary treatment
3. Smaller size of the inocula required
4. The process produces very less volume of sludge
5. The process is easy to operate and maintain

ABBREVIATIONS

COD:	Chemical Oxygen Demand
HRT:	Hydraulic Retention Time
GIDC:	Gujarat Industrial Development Corporation
TS:	Total Solids
TDS:	Total Dissolved Solids
TSS:	Total Suspended Solids
HPC:	High Plate Count Agar
CFU:	Colony Forming Unit
OD:	Optical Density
nm:	Nanometer
rpm:	Revolutions Per Minute
ID:	Internal Diameter
mg/L:	Milligram Per Litre
RBC:	Rotating Biological Contactor

ACKNOWLEDGEMENT

Authors gratefully acknowledge the management and staff of the Ankleshwar Research and Analytical Infrastructure Ltd. (ARAIL), Ankleshwar, Gujarat for the support and facilities provided to carry out this work.

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How to Cite this Article: Shukla Y. V., Pansuriya H. G., Characterization and Treatment of Industrial Effluents Using Adapted Consortium in Fixed Film Bioreactor. *Indian J Microbiol Res* 2015;2(2):95-105.