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Research Article

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Treatment of Sugar Mill Effluent by Biocontrol Agents (Trichoderma harzianum and Pseudomonas fluorescens) and its Effect on the Growth Parameters of Chick Pea

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ABSTRACT

This study was carried out to analyze Physicochemical parameters like Colour, odour, pH, electrical Conductivity (EC),Total suspended solids (TSS), Total dissolved Solids (TDS), Dissolved Oxygen (DO), Biochemical Oxygen Demand(BOD), Chemical Oxygen Demand (COD), Hardness, Sulfatesand Chloride of untreated sugar mill effluent and to degrade the effluent using biocontrol agents Trichoderma harzianum and Pseudomonas fluorescens. In the present study the effect of effluent (uninoculated) on seed germination at various concentrations and growth (inoculated) of chick pea was studied. The results of the parameters analysis revealed that untreated sugar mill effluent was green black in colour with offensive odour. pH was low with high organic load such as EC, TSS,TDS, BOD and COD which were higher than the permissible limit. The results of the degradation study shows that fungus Trichoderma harzianum and bacterium Pseudomonas fluorescens were found to be very much effective in reduction of pollutants (selected parameters). Decolorization results also reveal reduction color intensity with the incubation time. Trichoderma harzianum was more effect at the reduction percentage range of 20-88% whereas Pseudomonas fluorescens showed reduction percentage range of 16-83 %. Germination studies was conducted with chick pea seed treated with different concentrations (control, 10,25,50,75 and 100%) of sugar mill effluent. Germination decreased with the increase of effluent concentrations (25-100%).Growth parameters such as shoot length, root length,dry weight of seedlings were found to be increased in comparision to control.

Keywords: Physico chemical; Biodegradation; Biocontrol agents; Decolorization; Germination

INTRODUCTION

Pollutants and recalcitrant are becoming a major concern to global environment [1]. Sugar industry is one of the most important agro based industries in India and is highly responsible for creating significant impact on rural economy in particular and countries economy in general. Sugar industries rank second among the agro based industries in India. Sugar industry is seasonal in nature and operates only for 120 to 200 days in a year. A significant large amount of waste is generated during the manufacture of sugar, [2, 3].There are about 453 sugar mills in India, potentially producing about 287.4 million tonnes (2008-2009) of sugar per year. Most of the sugar mills are located in Uttar Pradesh, Maharashtra, Tamil Nadu, Gujarat, Karnataka and Andhra Pradesh. Effluent emanating from sugar industries is dark colored, acidic, with high biological oxygen demand, chemical oxygen demand, and liquid consisting of biodegradable organic and inorganic constituents, which cannot be disposed directly into water bodies [4].

Bioremediation is the term use to degrade or detoxify environmental contaminants by microorganisms, is among the new research technologies. This is environment friendly and cost effective technique [5].

MATERIALS AND METHODS

Collection of Effluent

The effluent sample was collected in sterile plastic containers from Sugar industry located in Uttar Pradesh, India. It was preserved by adding chemicals to analyze in the laboratory. The samples were withdrawn from direct outlet out following the waste water into the tank and from the treatment tank.

Analysis of Physicochemical Characteristics of Sugar Mill Effluent Effluent

The effluent sample was analysed for various physicochemical and biological parameters. The analysis was performed according to the APHA guidelines [6]. pH, Temperature, Color, Total Hardness, Conductivity, Total Dissolved solids, Total suspended solids, Sulphates, DO, BOD, COD and Chlorides were measured.

Cultures used

The biocontrol agents used for the bioremediation of sugar mill effluent *Pseudomonas fluorescens* and *Trichoderma harzianum*.

Biodegradation of Sugar Mill Effluent Using Pure Cultures

The pure cultures of biocontrol agents *Trichoderma harzianum* and *Pseudomonas fluorescens* were obtained from Microbiology Laboratory of Biotech Park, Lucknow, India and used for biodegradation of industrial effluent by following the procedure of Noorjahan with some modifications [7]. Effect of microbes on Color, BOD, COD, Sulfates, TDS and Hardness removal from the effluent was studied. Accurately 250 ml of effluent was taken into 500 ml conical flask and sterilized in autoclave at 121°C temperature and 15 lbs psi pressure for 15 minutes. To each flask, 1ml of appropriate inoculums of selected bacterial culture and fungal culture was added and were incubated at 32°C. Samples were taken out at different time intervals and the solutions were estimated for reduction of parameters. The Percentage reduction was calculated by difference in untreated and treated effluent.

Decolorization

The absorbance at 475 nm is commonly used as standard index to determine molasses pigment decolorization [1]. Melanoidin decolorizing activity was measured as decrease in optical density or absorbance at 475 nm. Samples were drawn at different intervals (at 7 day, 15 day, 22 day and 30 day incubation). Samples were filtered and centrifuged at 5000 rpm for 10 minutes and optimal density of supernatent measured in Spectrophotometer (Labtronics) at 475 nm as a decolorizing of melanoiden and other coloring agent in effluent.

Effect of Sugar Mill Effluent (inoculated with microbes) on Seed Germination and Growth of Chick Pea

Seed Germination

Seeds of chickpea were sterilized with 0.1% w/v mercuric chloride solution for 5 minutes to remove microbes and then washed three times with sterile distilled water. Seed germination was observed in glass dishes on sterile cotton pad with covered lid. The effluent was diluted to 0, 10, 25, 50, 75 and 100% with distilled water. These various concentrations (10, 25, 50, 75 and 100%) of sugar mill effluent solution were used for both germination and growth studies. Germination percent was calculated from the following formula.

$$\text{Germination percentage} = \frac{\text{No of seeds germinate}}{\text{Total No of seeds grown}} \times 100$$

Effect on Growth

Soil was sterilized 3 times to remove microbes and filled in plastic tray.100 ml of effluent in two 250 ml each were sterilized. They were inoculated with *Trichoderma* and *Pseudomonas* separately. Flasks were incubated for 3 days. The cultured broth was diluted and earlier germinated seeds were treated for growth studies. Ten seeds were sown at 5 cm. depth in 250 g sterilized soil containing plastic pot. Control was also maintained without inoculated seed. After 22 days chick pea plant were harvested. Shoot and root length were recorded in cm of each plant. Then plants were dried in oven at 65 °C for three days. After this dry weight were recorded in g.

RESULTS AND DISCUSSION

Results of the physicochemical parameters of untreated sugar mill effluent are given in Table 1.The results of the analysis revealed that colour of the untreated industrial effluent were green blackish with unpleasant odour. This colour and odour could be due to presence of organic and inorganic matter [8]. A large number of pollutants can impart colour, taste and odour to the receiving water, thereby making them unaesthetic and unfit for domestic consumption [9,10]. Color is mainly due to the presence melanoidin. In the present study temperature of the untreated effluent was recorded as 32°C. pH of the effluent was found 4.8 indicating it highly acidic. Electrical conductivity was found 0.990 ms/cm Among the analysed parameters Total Dissolved Solid (TDS) and Total Suspended Solid (TSS) were found 3700 mg/l and 320 mg/l respectively. Dissolved Oxygen (DO) was found 0.5 mg/l. B.O.D and C.O.D were found 3000 mg/l and 4800 mg/l respectively. Chloride was 100.11 mg/l and sulfate 63.4 mg/l. Hardness was 1950 mg/l.

Table 1: Results of the physicochemical parameters of untreated sugar mill effluent

Parameters	Wastewater	Limit (C.P.C.B)
Color	Green blackish	Colourless
Odour	Decaying molasses smell	Odourless
pH	4.8	5.5-9.0
D.O (mg/l)	0.5	6
T.D.S (mg/l)	3700	2100
T.S.S (mg/l)	320	100
EC (ms/cm)	990	-
BOD (mg/l)	3000	100 (for disposal on land) 30 (for disposal in surface waters)
COD (mg/l)	4800	250
Cl (mg/)	100.11	600
Sulfates (mg/)	63.4	1000
Hardness (mg/)	1950	-

Biodegradation of sugar mill effluent using bacterium *Pseudomonas flourescens* and fungus *Trichoderma*

Pure culture of bacterium, *Pseudomonas fluorescens* and fungus, *Trichoderma harzianum* were used for biodegradation of sugar mill effluent. Analysis of degradation of effluent using *Pseudomonas fluorecens* and *Trichoderma harzianum* are presented in Figures 1-5.

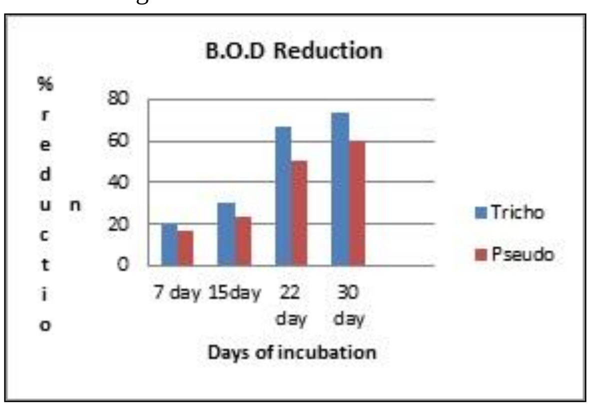

Figure 1: Variation of B.O.D after treatment with Trichoderma and Pseudomonas

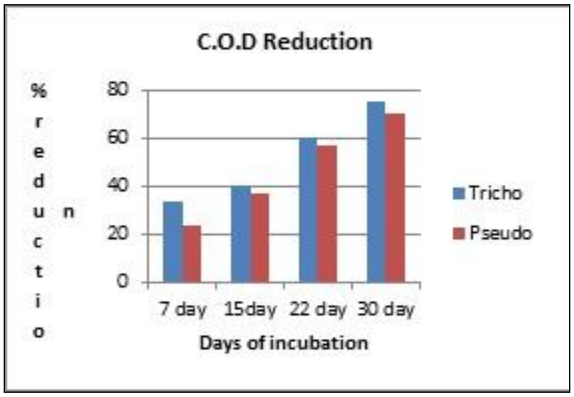

Figure 2: Variation of C.O.D after treatment with Trichoderma and Pseudomonas

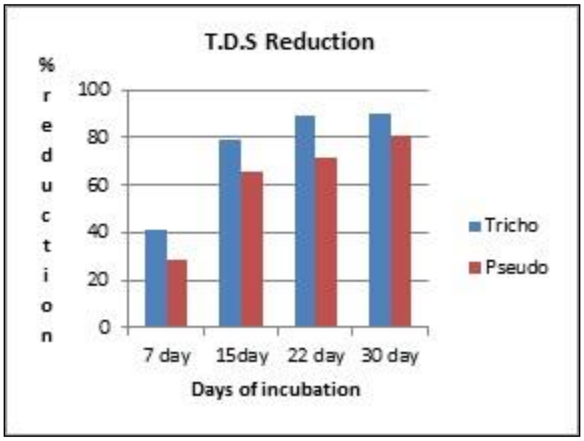

Figure 3: Variation of T.D.S after treatment with Trichoderma and Pseudomonas

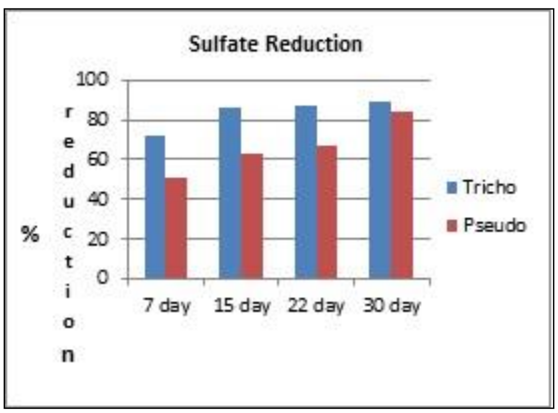

Figure 4: Variation of sulfate after treatment with Trichoderma and Pseudomonas

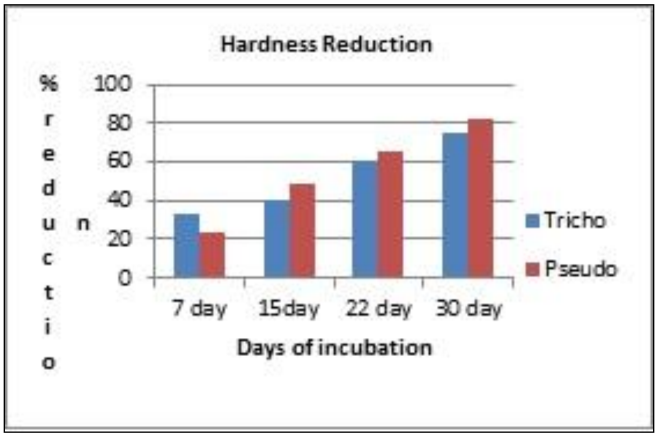

Figure 5: Variation of T.D.S after treatment with Trichoderma and Pseudomonas

BOD of industrial effluent before treatment is 3000 mg/l which is beyond the permissible limit (30 mg/l) of CPCB [11] for disposal. Samples were drawn to observe effect of degradation. *Trichoderma* treated BOD at 7 days of incubation is 2400 mg/l and the percentage reduction is 20%, B.O.D at 15 days of incubation is 2100 mg/land the percentage reduction is 30%, BOD at 22 days of incubation is 1000 mg/land the percentage reduction is 66.6%, BOD at 30 days of incubation is 800 mg/land the percentage reduction is 73.3%. Pseudomonas treated BOD at 7 days of incubation is 2500 mg/land the percentage reduction is 16.6%, BOD at 15 days of incubation is 2300 mg/land the percentage reduction is 23.3%, BOD at 22 days of incubation is 1500 mg/l and the percentage reduction is 66.6%, BOD at 30 days of incubation is 1200 mg/l and the percentage reduction is 60%.

COD of the effluent before treatment is 4800 mg/l. *Trichoderma* treated COD at 7 days of incubation is 3226 mg/l and the percentage reduction is 33.3%, COD at 15 days of incubation is 2880 mg/l and the percentage reduction is 40%, COD at 22 days of incubation is 1760 mg/l and the percentage reduction is 63.3%, COD at 30 days of incubation is 1253 mg/ l and the percentage reduction is 73.8%. *Pseudomonas* treated COD at 7 days of incubation is 3680 mg/l and the percentage reduction is 23.3%, COD at 15 days of incubation is 3040 mg/l and the percentage reduction is 36.6%, COD at 22 days of incubation is 2080 mg/l and the percentage reduction is 56.6%, COD at 30 days of incubation is 1440 mg/l and the percentage reduction is 70%.

TDS of effluent before treatment is 3700 mg/l. Trichoderma treated TDS at 7 days of incubation is 2180 mg/l and the percentage reduction is 28.10%, TDS at 15 days of incubation is 760 mg/l and the percentage reduction is 79.45%, TDS at 22 days of incubation is 400 mg/l and the percentage reduction is 89.18%, T.D.S at 30 days of incubation is 360 mg/l and the percentage reduction is 90.29%. Pseudomonas treated TDS at 7 days of incubation is 2660 mg/l and the percentage reduction is 28.10%, TD. at 15 days of incubation is 1260 mg/l and the percentage reduction is 65.94%, TDS at 22 days of incubation is 1040 mg/land the percentage reduction is 71.89%, TDS at 30 days of incubation is 720 mg/l and the percentage reduction is 80.54%.

Hardness of effluent before treatment is 1950 mg/l. Trichoderma treated hardness at 7 days of incubation is 1550 mg/l and the percentage reduction is 20.5%, hardness at 15 days of incubation is 1000 mg/l and the percentage reduction is 48.7%, hardness at 22 days of incubation is 675 mg/l and the percentage reduction 65.3%, hardness at 30 days of incubation is 335 mg/l and the percentage reduction is 82.8%. Pseudomonas treated hardness at 7 days of incubation is 1650 mg/l and the percentage reduction is 15.38 %, hardness at 15 days of incubation is 1200 mg/l and the percentage reduction is 38.46%, hardness at 22 days of incubation is 800 mg/l and the percentage reduction is 58.97%, hardness at 30 days of incubation is 550 mg/l and the percentage reduction is 71.79%. Sulfates in effluent is 63.4.Trichoderma treated sulfates at 7 days of incubation is 17.546 mg/l and the percentage reduction is 72.3%, at 15 days of incubation is 8.920 mg/l and the percentage reduction is 85.9%, at 22 days of incubation is 8.168 mg/l and the percentage reduction is 87.11%, hardness at 30 days of incubation is 7.023 mg/l and the percentage reduction is 88.91%. Pseudomonas treated sulfates at 7 days of incubation is 31.441 mg/l and the percentage reduction is 50.40%, at 15 days of incubation is 23.577 mg/l and the percentage reduction is 62.81%, at 22 days of incubation is 20.691 mg/l and the percentage reduction is 67.49%, at 30 days of incubation is 10.391 mg/l and the percentage reduction is 83.67%. This work is supported by Noorjahan CM. [7].

Decolorization

The effluent color is green blackish before degradation but after degradation using *P.flourescens* and *Trichoderma harzianum.* There is a change in color from green blackish to light yellow (*P.flourescens)* and green black to almost colourless (*Trichoderma harzianum*) of the sugar mill effluent. The odour of effluent was offensive in nature having molasses decaying smell before degradation but after degrading the effluent using *P.flourescens* and *Trichoderma harzianum* samples shows odourless condition. This may be due to the action of microbes which decomposed the melanoiden content and toxic pollutants present in the effluent and made the change in color and odour of the effluent. This is supported by the work of Sukumaran et al, [12].

Decolorization activity is measured by taking the absorbance at 475 nm. The absorbance of treated sample was subtracted from the absorbance of untreated absorbance and percentage was determined. Reduction in absorbance indicating decolorizing activity is represented in Figure 6. Trichoderma treated absorbance at 7 days of incubation is 0.0469 and the percentage reduction is 30.51 %, at 7 days of incubation is 0.0469 and the percentage reduction is 30.51 %, at 15 days of incubation is 0.0364 and the percentage reduction is 46.07 %, at 22 days of incubation is 0.0287 and the percentage reduction is 57.48 %, at 30 days of incubation is 0.0200 and the percentage reduction is 70.37%. Pseudomonas treated absorbance at 7 days of incubation is 0.0585 and the percentage reduction is 13.3 %, at 15 days of incubation is 0.0488 and the percentage reduction is 27.70 %, at 22 days of incubation is 0.0430 and the percentage reduction is 36.29 %, at 30 days of incubation is 0.0330 and the percentage reduction is 51.11 %. This is supported by the work of Lakshmi Anil kumar [13].

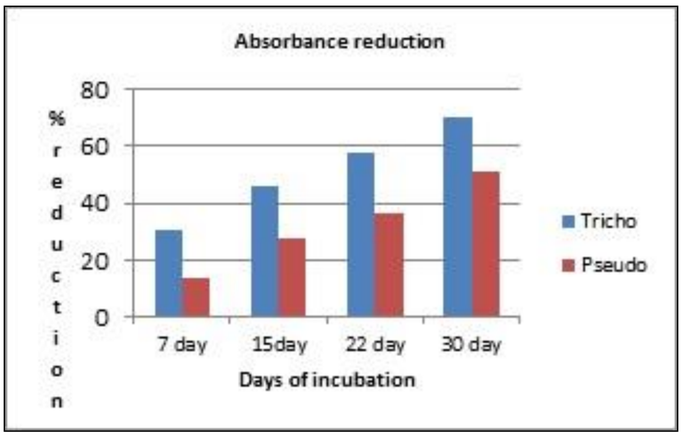

Figure 6: Variation in absorbance after treatment with Trichoderma and Pseudomonas effect of sugar mill effluent (inoculated with microbes) on seed germination and growth of chick pea

Germination percentage of seeds varied with respect to different concentrations of effluent (Tables 2-4). The percentage of seeds germinating decreases as the effluent concentration increases. The percentage germination and germination value was maximum at the lower effluent concentrations of 10% and 25%.

Table 2: Results of germination percentage at different concentration

EffluentConcentration	% germination
Control (100%)	90
10%	90
25%	80
50%	76
75%	60
100%	50

At lower concentration (10% - 25%) of effluent germination percentage is higher, 90% - 80% of the seeds germinated. The effluent contained high amount of plant nutrient which may minimize use of fertilizer to promote development of plant [14]. The sugar mill effluent used in developing countries for agriculture as fertilizer has gained more significance as it is considered as a source of organic matter and plant nutrients. Root length, Shoot length and dry weight of treated seed show increase in length and weight in comparision to control.

Table 3: After 22 days shoot and root length (cm-)

Plant	Control		*Trichoderma harzianum* treated effluent		*P.fluorescens* treated effluent	
	Shoot	Root	Shoot	Root	Shoot	Root
1	5.9	4.1	15.4	10	13.5	9.5
2	5.1	2.9	15.4	9.5	17.7	9.7
3	3.5	4.5	15.8	11	12	8
4	15.2	2.4	12.5	8.4	13.7	7.5
5	11.8	7.1	14.6	8.7	13	9
6	13.5	6	12.2	5.1	11.5	8.5
7	12.5	4.5	10.8	6.3	13.5	9
8	12	7.4	16	6.6	15.7	9.2
9	14.8	7.2	14.2	5.2	12.8	9.2
10	9.7	7.6	13	8.2	15.3	4.4
Total	104	53.7	139.9	79	138.7	84
Mean	10.4	5.37	13.99	7.9	13.87	8.4

Table 4: After 25 days dry weight of harvested plants in grams-

Plant	Control	*Trichoderma harzianum* treated effluent	*P.fluorescens* treated effluent
1	0.0723	0.08425	0.09889
2	0.0978	0.11461	0.13358
3	.09248`	0.16769	0.13802
4	0.04965	0.10494	0.10687
5	0.07136	0.14509	0.15283
6	0.09848	0.13252	0.15881
7	0. 09234	0.14211	0.12309
8	0.0794	0.09637	0.11408
9	0.0808	0.08857	0.1066
10	0.09339	0.0679	0.1254
Mean	0.828	1.14405	1.09936

CONCLUSION

From the above investigation, it can be inferred that the maximum reduction of physico- chemical parameters was recorded in treated sample using native fungus *Trichoderma harzianum* compared to *Pseudomonas fluorescens* treated effluent.The sugar mill effluent which is untreated contains high COD, BOD, TSS, TDS, TS and low contents of DO which is beyond the permissible limit of disposable and not suitable for irrigation.Thus from the foregoing discussion it is very clear that microbes play an important role in the biodegradation of organic and inorganic matter. They have enzymes that allow them to use environmental contaminants as food and hence make them ideal for biodegradation. *Trichoderma harzianum* and *Pseudomonas fluorescens* are agriculturally important microbes and biocontrol agents. From the present study, *Trichoderma harzianum* and *Pseudomonas fluorescens* showed efficient degrading capabilities by degrading the contaminants as they use it for their growth and reproduction. Organic and inorganic compounds are a source of the toxic organic contaminant is converted to innocuouschemical compound, obtain energy by catalyzing energy producingchemical reactions and this energy is used in the production of new cells [15]. Lastly both organisms may be used for biodegradation and biodegraded waste water containing these beneficial microbes may serve for soil health and plant disease control.

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Conflict of Interest

Authors declared no conflict of interest.

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