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Treatment of sugar mill effluent by microbes and its effect on the growth parameters of pulses

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Abstract: The sugar mill effluent discharging industries are sugar mill dyeing, textile, printing, tanneries, pharmaceuticals, distillers, detergents, chemical factories, paper mills, fertilizer factories, etc. The present study was undertaken analyze the physio-chemical parameters, isolation and identification of microbes (bacteria and fungi) and decolorization of sugar mill effluent. The physio chemical parameters such as alkalinity, free CO_2, Total CO_2, calcium chloride, BOD, COD, DO, ammonia, nitrate, nitrite, inorganic phosphate and organic carbon in both treated and untreated samples were analysed. Various microorganisms were isolated and identified from (bacteria and fungi) sugar mill effluent. The sugar mill effluent used to cultivate the 3 types of pulses *i.e*., ground nut, cow pea and black gram of germination and seedling growth. *Azotobacter* spp. and *Beauveria bassiana* was decolourized with sugar mill effluent for better low cost, production of biofertilizer and biocontrol agents. The following concentrations were used in seed germination and seedling growth (treated and untreated) namely, Control, 50%, 75%, 100%. The treated effluent was recommended to cultivable land. In our findings, *Azotobacter* spp. and Be*auveria bassiana* treated effluent was well decolourized and the seed germination and seedling growth showed well compared with *Beauveria bassiana* and control.at dissipation

Key words: Sugar mill effluent, *Azotobacter* spp. *Beauveria bassiana*

Introduction

The sugar mill effluent discharging industries are sugar mill dyeing, textile, printing, tanneries, pharmaceuticals, distillers, detergents, chemical factories, paper mills, fertilizer factories, etc. Mycological examination of effluent water is necessary for determining it fitness for use of agricultural irrigation. Such sugar can contain 70% of water, 14% of fiber, and 13.3% of saccharose (about 10-15% sucrose) and soil impurities (World Bank, 1995). The sugar industrial effluents were toxicity to aquatic fauna and human health. The pH of effluent is 4 and observed concentration is 43,000 mgl^{-1} for Biological Oxygen Demand (BOD) and 89,760 mgl^{-1} for Chemical Oxygen Demand (COD). These are beyond the tolerance limit of the water causing shifting of the algal forms towards more tolerant zone leading to decrease in biodiversity. Total solids, Total Dissolved Solids, and Suspended solids were also considerably high (Matkar *et al.,* 2002).

Azotobacter spp. is gram negative bacteria, pleomorphic *i.e*. they are of different size and shapes. Their size ranges from 2-10x1-2.5m., the species of *Azotobacter* spp. are known to fix an average 10mg of N/g of sugar in pure culture on a nitrogen free medium. *Beauveria bassiana* is a fungus that grows naturally in soils throughout the world and acts as a parasite on various arthropod species, causing white muscardine disease; it thus belongs to the endomopathogenic fungi. It is being used as a biological insecticide to control a number of pests such as termites, thrips, whiteflies, and different beetles (Donald, 2005). Cow peas are one of the most important food legume crops in the semi-arid tropics covering Asia, Africa, Southern Europe, Central and South America. It also has the useful ability to fix atmospheric nitrogen through its root nodules, and it grows well in poor soils with more than 85% sand and with less than 0.2% organic matter and low level phosphorus (Singh, 2003). Black Gram is a highly valued pulse crop. It is a very prominent member of the white spread Asiatic *Vigna* crop family. Black Gram is a perfect combination of all nutrients which includes 20 to 25% proteins, 40 to 47% starch, ash, fats, carbohydrates and essential vitamins. Black Gram has all the essential nutrients which it makes to turn in to a fertilizer. *Hypogea* means "under the earth", after pollination, the flower stalk elongates causing it to bend until the ovary touches the ground. Continued stalk growth the pulses the ovary underground where the mature fruit develops into a legume pod, the peanut. Pods are 3 to 7 cm long, containing 1 to 4 seeds.

The present research work has been carried out with an aim, Collection of sugar mill effluent and analysis of physio- chemical properties, Isolation and Identification of microbes from sugar mill effluent, Decolorization the sugar mill effluent by using *Azotobacter* spp. and *Beauveria bassiana* and to explore the germination and seedling growth of some pulse seeds (before and after treatment).

Materials and methods

Collection of sampleMa: The effluent sample was collected in sterile bottle from Aringnar Anna Sugar mill located at Kurungulam, Thanjavur district, Tamil Nadu. South India.

Isolation and identification of microbes: The bacteria and fungi were isolated from the effluent by serial dilution method 10^{-7}, 10^{-8} and 10^{-3} 10^{-4} (Grant *et al*., 1971). The isolated organisms were identified by various biochemical methods by James (1958) and Barnett, (1972). The fungal culture were identified using Lacto phenol cotton blue staining (Gilmann, 1957).

Treatment of sugar mill effluent: Treatment of sugar mill effluent was 300ml of sugar mill effluent was taken in 500ml of conical flask at four numbers in two set, one is control in each set. In first set three flasks were inoculated with *Azotobacter* spp and another set of flasks were inoculated with *Beauveria bassiana.* Then the flasks were incubated in laboratory shaker at 37^0 C for 15 days for degradation of sugar mill effluent (Pandey, 2007).

Physio-chemical properties of sugar mill effluent: The physio – chemical parameters were analyzed from sugar mill effluent by APHA , 2005.

Seed germination and seedling growth of pulse variety by using sugar mill effluent (before and after treatment): The sugar mill effluent was used to study the effect of seed germination and seedling growth on pulse variety. The one set of effluent was untreated and another two set of effluent was treated by *Azotobacter* spp. and *Beauveria bassiana* separately. The following concentrations were used in seed germination and seedling growth of the three pulse variety like (*Vigna mungo* (Black Gram), *Arachis hypogaea* (Ground nut) and *Vigna unguiculata* (cow pea)) were selected for sugar mill effluent treatment (Before and after treatment) C – Control, T1 - 50% of sugar mill effluent, T2 - 75% of sugar mill effluent and T3 -100% of sugar mill effluent. The morphometric analysis were observed and recorded up to 15 days of plant growth.

Results and Discussion

Physio-chemical properties of sugar mill effluent: The present study was used to treat the sugar mill effluent by two microbes like *Azotobacter* spp. and *Beauveria bassiana.*. Free CO_2 and Total CO_2, Calcium, BOD, COD, Ammonia, Nitrate, Nitrite were reduced and total CO_2, alkalinity were increased during the process of decolourization of the sugar mill effluent. Physio-chemical properties of sugar mill effluent (before and after treatment) were analyzed (Table 1).

The sugar industrial effluents toxicity to aquatic fauna and human health. The pH of the effluent is 4.00 and the concentration is 43000 mgl^{-1} for BOD and 89,760 $mgl^{-1}$for COD. These are beyond the tolerance limit of the water causing shifting of the algal forms towards more tolerant zone leading to decrease in biodiversity. Total solids, total dissolved solids and suspended solids were also considerably high discussed by Matkar *et al*. (2002).

The treatment of sugar mill wastewater was studied using a pilot up flow anaerobic sludge blanket (UASB) reactor. The effluent was a molasses-based substrate with a chemical oxygen demand (COD) of about 1000 $mgl^{-1.}$ After a successful start-up of the reactor, results showed that with a hydraulic retention time (HRT) at or above four hours and with an average organic loading rate (OLR) below 6.7 kg COD m^{3} /Day the COD removal efficiency of the system was over 76% was discussed by Ragen *et al*., (2005).

Isolation and identification of microbes: In the present study, isolation and identification of some bacterial and fungal strains were done. Bacteria like *Pseudomonas, Enterobacter aerogens, Streptococcus* spp. *Proteus vulgaris* and *Staphylococcus* spp. were isolated. Totally seven species of fungi were isolated from the sugar mill effluent and identified based on colony morphology, spore characters. The identified fungi such as *Aspergillus sydowi, Aspergillus terreus, penicillium citrinum, penicillium chrysogenum, Rhizoctonia oryzae, Rhizopus nigricans* and *Trichoderma harzianum*. Microbiological studies revealed the presence of specific fungal species which are capable of growing in high concentration of bicarbonate and nitrates which in turn serve as

Table - 1: Physio-chemical Analysis of Sugar mill effluent Before and After treatment

Parameters	Before Treatment	After treatment	
		Azotobacter	***B.bassiana***
Colour	Turbid black	Pale yellow	Pale yellow
Odour	Foul	Alcohol smell	Alcohol smell
pH	7.6	6.1	5.3
Alkalinity	127.5	49. 13	52.5
Free CO_2	114	97	96
Chloride	53.12	43.5	41.4
DO [a]	21.70	Nil	Nil
BOD [b]	18.6	21.5	18.3
COD [c]	102.4	78	81
Ammonia	4.3	3.1	2.7
Nitrate	11.3	7.8	6.1
Nitrite	0.463	0.390	0.335
Inorganic PO_4	1.50	0.950	0.780
Organic carbon	4.350	0.210	Nil

All values are in mgl^{-1} except pH , colour, odourwithout unit; Do[a]- Dissolved oxygen; BOD[b]- Biochemical oxygen Demand; COD[c]- Chemical oxygen demand

Table - 2: Effect of untreated sugar mill effluent on seed germination and seedling growth of pulses (before treatment)

Parameters	Treatment	Cowpea	Black gram	Groundnut
No of germination in %	C	100±0	90±3	60±4
	T1	90±3	90±2	60±3
	T2	90±2	90±2	70±3
	T3	80±4	100±0	40±5
Plant height(in cm)	C	8.25±1	9.10±2	5.10±1
	T1	8.18±1	9.50±1	5.67±2
	T2	8.42±2	9.00±3	4.92±1
	T3	8.47±1	7.70±5	1.82±2
No of leaves/plant	C	5.1±1	5.2±2	10.16±1
	T1	5.2±1	5.4±2	12.83±2
	T2	5.0±2	5.0±1	9.28±1
	T3	5.0±1	5.3±1	9.0±1
No of roots/plant	C	15±2	18±1	24±2
	T1	17±1	21±1	26±1
	T2	18±2	19±1	26±1
	T3	15±1	16±1	23±2
Root length (in cm)	C	11±1	11.5±1	9.0±2
	T1	12±2	13.0±1	11.0±1
	T2	10.5±2	12.5±1	12.0±1
	T3	10±1	12.0±1	10.0±1

Control- normal water, Treatment 1 – 50% effluent and 50% normal water; Treatment 2 – 75% effluent and 25% water; Treatment 3- 100% effluent; Values are Mean ± Standard deviation; Values are triplicates.

Table - 3: Effect of *Azotobacter* spp. treated sugar mill effluent on seed germination and seedling growth of pulses

Parameters	Treatment	Cowpea	Black gram	Ground nut
No of germination in %	C	90±3	90±4	100±0
	T1	100±0	100±0	80±5
	T2	100±0	100±0	70±4
	T3	90±4	100±0	100±0
Plant height(in cm)	C	8.25±1	9.4±1	6.10±2
	T1	9.78±2	11.5±1	9.20±1
	T2	10.30±1	10.00±1	10.5±1
	T3	10.00±1	9.5±1	9.1±1
No. of leaves/plant	C	4.5±1	5.2±2	10.16±1
	T1	5.0±1	5.9±2	11.9±1
	T2	5.3±1	5.0±1	12.28±1
	T3	4.7±1	4.9±1	10.50±1
No. of roots/plant	C	15±1	18±1	24±2
	T1	19±2	19±1	25±1
	T2	21±1	20±1	27±2
	T3	18±2	18±1	24±1
Root length (in cm)	C	12±1	12.5±2	10.0±1
	T1	16±1	14.5±2	12.0±1
	T2	17±1	13.5±1	13.0±2
	T3	15±1	12.0±1	11.0±2

Control- normal water, Treatment 1 – 50% treated effluent and 50% normal water; Treatment 2 – 75% treated effluent and 25% normal water. Treatment 3- 100% treated effluent. Values are Mean ± Standard deviation; Values are triplicates

indicator organism of such pollutants (Amathussalam *et al.,* 2002). A number of treatment methods are available to reduce such pollutants among them. The bio treatment is the most attractive simple environmentally acceptable and economically feasible treatment method (Black *et al*., 1994).

Seed germination and seedling growth of pulse variety by using sugar mill effluent (before and after treatment): In the present study deals with sugar mill effluent was used in seed germination and seedling growth of 3 pulses like groundnut, black gram and cow pea. In various percentage, Control, 50%, 75%

Table - 4: Effect of *Beauveria bassiana* treated sugar mill effluent on seed germination and seedling growth of pulses

Parameters	Treatment	Cowpea	Black gram	Groundnut
No of germination in %	C	100±0	100±0	100±0
	T1	80±2	80±2	100±0
	T2	70±5	90±3	90±3
	T3	100±0	100±0	50±5
Plant height (in cm)	C	7.50±1	9.2±2	4.3±1
	T1	8.53±2	10.5±1	5.67±2
	T2	8.49±1	8.7±2	4.52±1
	T3	7.10±2	7.70±1	3.2±1
No of leaves/plant	C	4±1	4±2	7.6±1
	T1	4±1	4±1	11.2±1
	T2	4.5±1	4±2	8.75±2
	T3	4±1	4±2	8±2
No of roots/plant	C	14±1	16±1	21±1
	T1	16±2	18±2	19±1
	T2	17±1	19±2	21±1
	T3	14±2	16±3	18±1
Root length (in cm)	C	10.5±1	10.5±1	10±3
	T1	14±2	11±1	11±2
	T2	12±1	16±3	13±3
	T3	12±1	12±3	10±1

Control- normal water. Treatment 1 – 50% treated effluent and 50% normal water; Treatment 2 – 75% treated effluent and 25% normal water. Treatment 3- 100% treated effluent. Values are Mean ± Standard deviation; Values are triplicates

and 100%. Before treatment of black gram and cow pea was well germination and seedling growth was normal compared with ground nut. In 100% treatment, the germination and seedling growth was affected. After treatment of sugar mill effluent by using *Azotobacter* spp. and *Beauveria bassiana* the germination and seedling growth was well compared with before treatment. The results were observed and recorded (Table 2 to 4). Germination is a critical stage which ensures reproduction and consequently controls the dynamics of population, so it is a critical test for the probable crop productivity (Radosevich *et al*., 1997).

Conclusion

The present investigation concluded that the *Azotobacter* spp. and *Beauveria bassiana* can be used as a decolourization of sugar mill effluent. *Azotobacter* spp. treated effluent was well decolorized and the seed germination seedling growth was well compared with *Beauveria bassiana* treated effluent and control. In future, sugar mill effluent is treating with help of *Azotobacter* spp. and the treated effluent are used to irrigation purpose of agricultural field. It can be suggested that the sugar mill effluent can be used for irrigation purpose after proper dilution with water, which will be one of the solutions for combating studies on growth performance and yield of crop and properties of soil are needed to the present findings.

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