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Study on bioremediated sugar industry effluent for Irrigation: An evaluative study on the biochemical attributes of *Vigna radiata* under laboratory conditions

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ABSTRACT

The increasing pace of industrialization in public and private sectors along with urbanization, population explosion and green revolution are reflected in varying degree of water, soil and air pollution. Environmental pollution has a great health hazard to human, animals and plants with local, regional and global implications. Sugar industry plays an important role in the rural economy of India by uplifting the livelihood and creation of employment. The byproducts of sugar industry are also used as raw materials in different industries. However sugar industries posed a great environmental impact upon the surrounding environment as it releases various pollutants including huge amount of effluent. In the present investigation, laboratory experiments were conducted to determine the comparative phytotoxic effects of untreated and bioremediated sugar industry effluent with immobilized Spirogyra sp. The present research work includes the effects of different concentrations viz., 25%, 50%, 75% and 100% of sugar industry wastewater and bioremediated wastewater on percentage of seed germination, shoot length, root length, and some biochemical parameters viz., chlorophyll, protein, total carbohydrates, starch and free amino acids of Vigna radiata. The results of this research work revealed that, using undiluted effluent no seed germination was observed even after 7 days while in 75% diluted effluent 90% seeds were germinated after 7 days. While in bioremediated waste water, 100% seed germination was observed only after 3 days. The shoot length, root length and biochemical parameters showed inhibitory effects with increasing concentration of effluent. In case of bioremediated wastewater, strikingly most of the parameters showed stimulatory effects as compared to control.

Keywords : Sugar industry, Wastewater, Bioremediation, *Spirogyra sp.*, Stimulatory effects, Inhibitory effects.

INTRODUCTION

Increasing urbanization and industrialization in the developing countries like India pose severe problems in collection, treatment and disposal of effluents. However sugar mill have a great environmental impact upon the surrounding environment. India is the largest producer of sugar in the world. There are more than 550 installed sugar industries in the country. Sugar industries generate about 1000 liters of wastewater for every tonne of sugar cane crushed. Because of high BOD content, sugar industry wastewater deplete dissolved oxygen content of water bodies rendering them unfit for both aquatic life and human uses. Sugar industries rank second among the agro based industries in India. Sugar industry is seasonal in nature and operates for 120 to 200 days in a year. According to the report, in 2013 – 14 around 3456 lakh tons sugar with 10881 tons of molasses was produced [1].

At present several biological treatment technologies for sugar industry effluent like aerobic as well as anaerobic were used. For biodegradable impurities, the natural choice is biological treatment, which could either be aerobic or anaerobic. Anaerobic treatment converts the wastewater organic pollutants into small amount of sludge and large amount of biogas as source of energy; whereas aerobic treatment needs external input of energy for aeration [2].

Water is essential to human health, economic growth, and environmentally sustainable development. Urban population growth combined with rapid agricultural and industrial development has not only increased the total demand of fresh water, but also increased waste into the watercourses that has started degrading the quality of the environment. This has made natural water scarcity problem even worse. It is because water resources have been exploited to their maximum capacity [3, 4], and more and more water will be needed to satisfy urban water demand in the coming decades [5].

In order to conserve the limited water resources and protect the environmental quality, wastewater reuse is becoming more attractive. It has become now an option to relieve the demand on fresh water and environmental pressure thus it will play an important role in future water utilization patterns [6, 7]. Wastewater reuse has several benefits. After proper treatment, wastewater can be used for different purposes, such as agricultural irrigation. It becomes a new source of water instead of traditional water sources to sustain the demand on fresh water supplies [8, 9].

Nutrients such as nitrogen (N) and phosphorous (P) in the effluent if treated and used properly, can act as fertilizer for crop production [10, 11]. The water body has a limited capacity to absorb pollutants without declining the water quality. Wastewater treatment aims to remove as many as possible pollutants and disease-causing agents to protect the environment and ensure public health. It also provides a suitable effluent for reuse [12]. Wastewater treatment is a multi-stage process. The traditional requirement for treating the raw wastewater involves the removal of organic materials, suspended solids, to make the pathogens more stable and to remove the dissolved pollutants [13].

Damage to crops due to sugar industry effluent entering in agricultural land is reported. Farmers using the sugar industry wastewater for irrigation found that, plant growth and crop yield were reduced and soil health was compromised. Sugar industry effluents are commonly used for irrigation, thus it become essential to determine how crops respond when exposed to industrial effluents. In this regard efforts have been made to determine the effects of untreated and bioremediated sugar industry effluent on seed germination, shoot length, root length and biochemical parameters of *Vigna radiata*.

MATERIALS AND METHODS

Bioremediation studies
Bioremediation study was performed in 250 ml conical flasks containing 100 ml of effluent sample and 10 ml of immobilized beads of algal cells. The flask was incubated for 10 days at room temperature. The samples were collected after 10 days.

Experimental design for germination test
For germination tests, 10 seeds of *Vigna radiata* were placed in sterilized glass Petri dishes of uniform size lined with two filter paper discs. These discs were moistened with 5 ml of distilled water for control, with the same quantity of various concentrations of sugar industry effluent (25.0, 50.0, 75.0 and 100%) in distilled water and bioremediated samples. The Petri dishes were incubated at 30±1^{o}C in an incubator. Germination was recorded daily at a fixed hour, and the emergence of the radical was taken as a criterion of germination.

Phytotoxicity study
The phytotoxicity studies was carried out at room temperature using *Vigna radiata* seeds followed by watering separately with 5 ml sample of effluent of various concentrations and 5 ml of treated effluent per day. A control set was also kept by using tap water.

Biochemical estimation
Chlorophyll content was estimated by Arnon’s method [14]. Total chlorophyll was calculated for each sample using the Arnon’s formula. Protein contents were estimated by the Lowry method using Bovine Serum Albumin (BSA) as

standard [15]. The total carbohydrate and starch contents were estimated by the Anthrone reagent method [16]. The total free amino acids were estimated by the Ninhydrin method [17].

RESULTS AND DISCUSSION

The percentage germination of *Vigna radiata* seeds varied with respect to different concentrations of effluent. The percentage of seeds germinating decreases as the effluent concentration increases. 100% seed germination was observed in bioremediated sugar industry wastewater as shown in figure 1. Some author conducted the laboratory experiments to study the effect of different concentrations (0, 6.25, 12.5, 25, 50, 75, and 100%) of textile effluents (untreated and treated) on seed germination(%), delay index, plant shoot length and root length, plant biomass, chlorophyll content and carotenoid of three different cultivars of wheat. The textile effluent did not show any inhibitory effect on seed germination at low concentration (6.25%). Seeds germinated in 100% effluents but did not survive for longer period [18].

Similarly, the experiment concluded that the distillery effluents contain various organic and inorganic nutrients and may have a beneficial effect on the crop yield [19]. Some data revealed that the effect of different concentrations (0, 5, 10, 15, 25, 50, 75% and undiluted) of distillery effluent (raw spend wash) on seed germination (%), speed of germination, peak value and germination value of some vegetable crops, *viz*., tomato, chilli, bottle gourd, cucumber and onion. It was concluded that the effect of the distillery effluent is crop specific and due care should be taken before using the effluent for pre-sowing irrigation purposes. The distillery effluent did not showed any inhibitory effect on seed germination of low concentration except in tomato. Irrespective of the crop species, at highest concentration (75% and undiluted) complete failure was observed for germination [20].

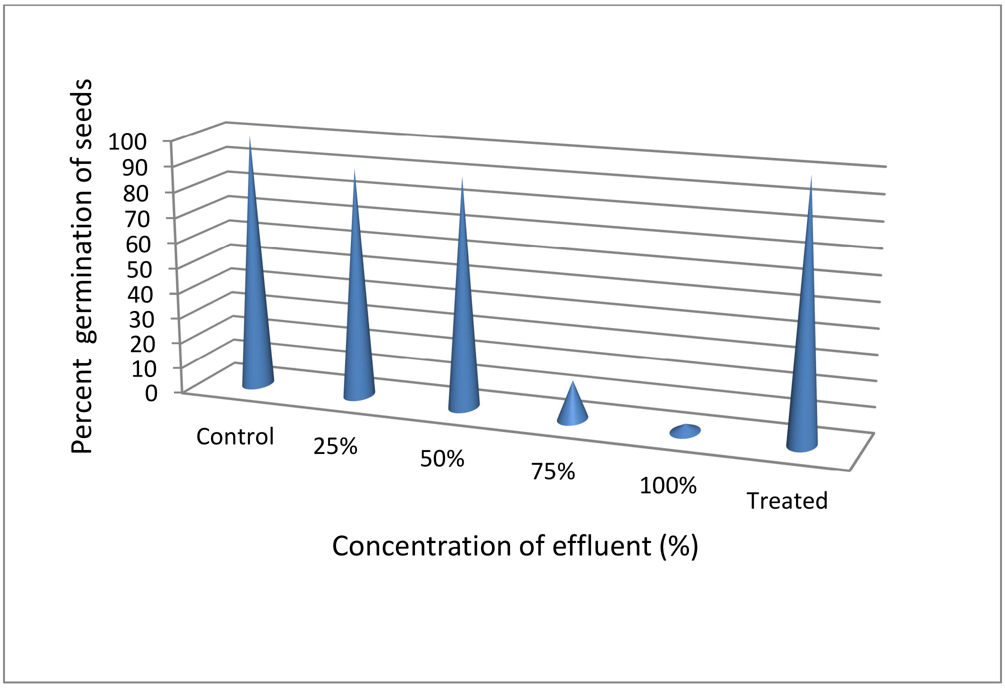

Figure 1. Effects of various concentrations and treated effluent of sugar industry on percent seed germination of *Vigna radiata*

The same pattern of seed germination was observed in shoot and root length and chlorophyll content of *Vigna radiata*. The shoot length and root length decreases as the effluent concentration increases as shown in figure 2. Both of these parameters of shoot and root length gives better results with bioremediated wastewater.

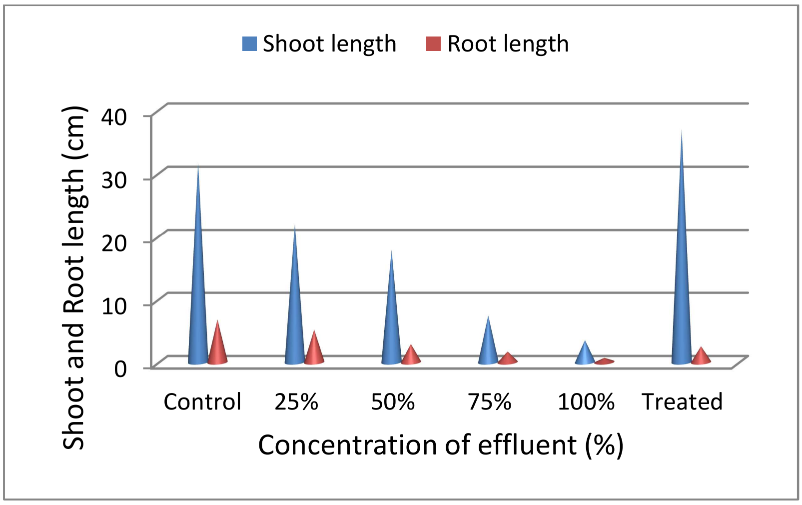

Figure 2. Effects of various concentrations and treated effluent of sugar industry on shoot and root length of *Vigna radiata*

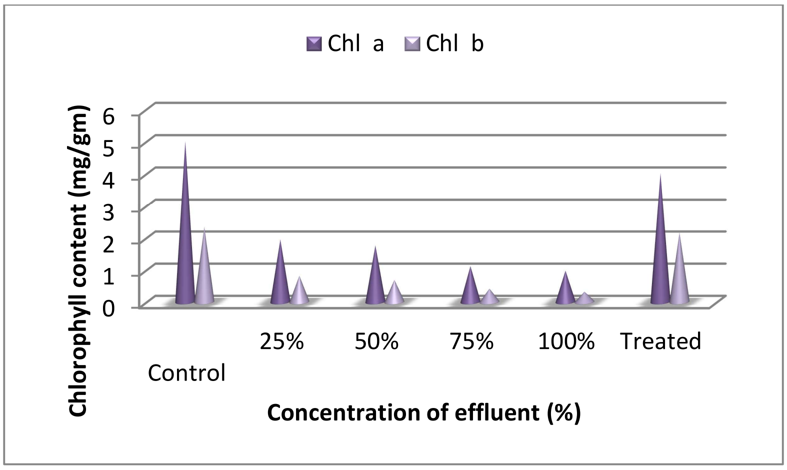

Figure 3. Effects of various concentrations and treated effluent of sugar industry on chlorophyll content of *Vigna radiata*

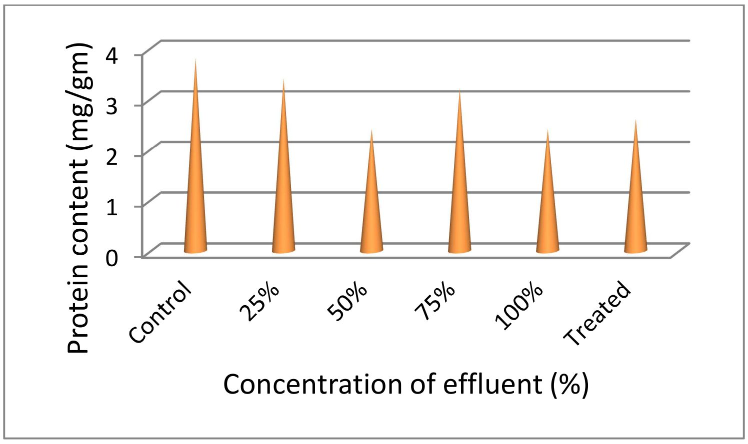

Figure 4. Effects of various concentrations and treated effluent of sugar industry on protein content of *Vigna radiata*

In the diluted and bioremediated effluent, pollution load of the effluent decreased. The effluent at lower concentration can serve as a liquid fertilizer for the cultivation of agricultural crops. The biochemical parameters like chlorophyll, protein, carbohydrate, starch and amino acids showed stimulatory effects when diluted and treated wastewater was used as shown in figure 4, 5, 6 and 7. The biochemical profile of water hyacinth prior to and after its growth in the aqueous solution of selenium showed adverse effect on chlorophyll a, chlorophyll b, total chlorophyll, protein, carbohydrate, starch and free amino acids. Selenium showed stimulatory effects at 5 mg/L and 10 mg/L for all the observed parameters. However selenium showed adverse effects at 20 and 50 mg/L concentrations for all the observed parameters [21].

Several researchers studied the effects of metal concentrations on chlorophyll, protein, carbohydrate, starch, and amino acid. Their study revealed that, the inhibitory and stimulatory effects of the used metals depend on concentration of the metals. Different organisms, however, have different sensitivities to the same metal, and the same organism may be more or less damaged by different metals [22, 23].

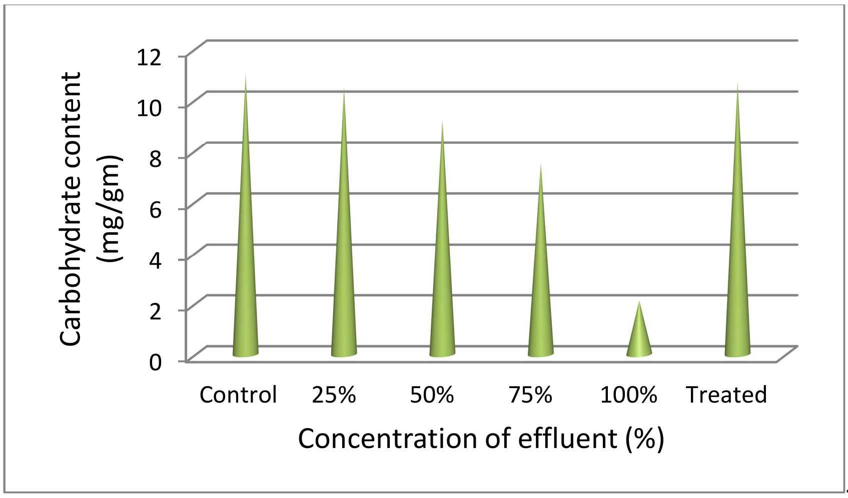

Figure 5. Effects of various concentrations and treated effluent of sugar industry on carbohydrate content of *Vigna radiata*

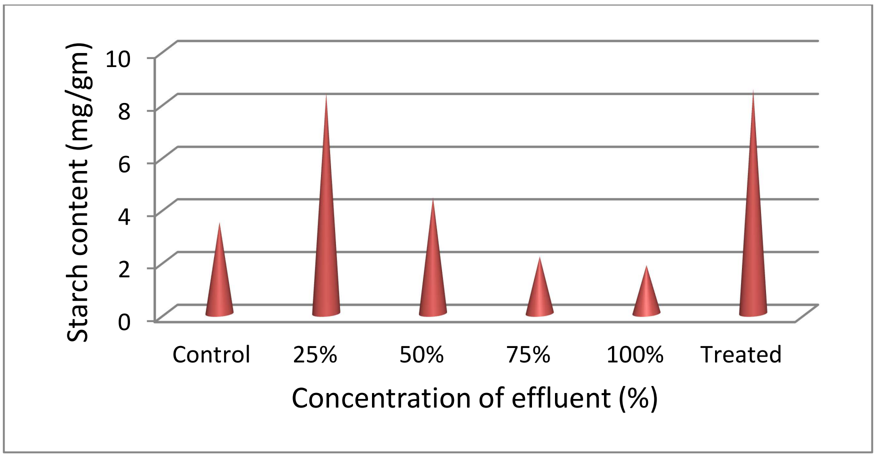

Figure 6. Effects of various concentrations and treated effluent of sugar industry on starch content of *Vigna radiata*

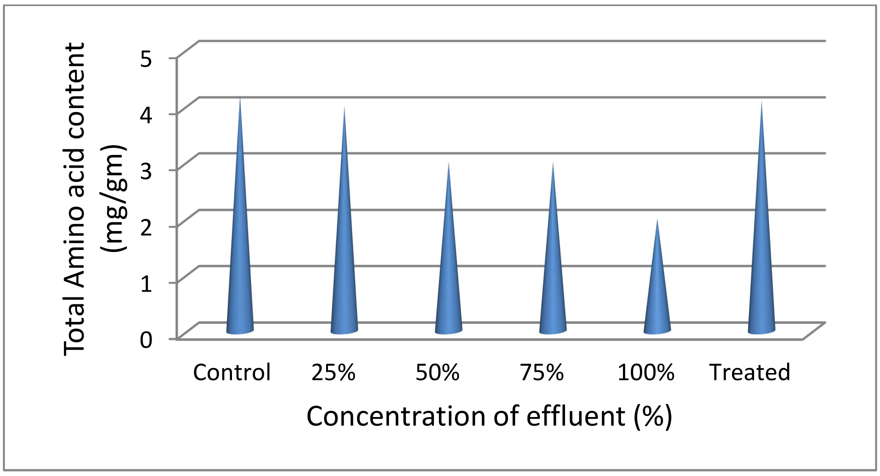

Figure 7. Effects of various concentrations and treated effluent of sugar industry on total amino acids content of *Vigna radiata*

The germination, growth and biochemical parameters of crop plants were inhibited at higher concentrations of effluent may be due to presence of toxic substances and osmotic pressure of higher dose, which makes inhibitions more difficult and reduce oxygen uptake by the seedlings of crop plants which is very important for their growth [24, 25, 26, 27].

CONCLUSION

The use of bioremediated sugar industry wastewater in crop plant nourishment would be beneficial alternative resources to fresh water. On the overall basis performance as exhibited by *Vigna radiata* when subjected to bioremediated effluent, it can be suggested that, treated sugar industry wastewater is a prospective source of different plant nutrients. Thus, bioremediated sugar industry effluent can be used for irrigation purpose in agricultural practices.

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