RESEARCH ARTICLE

PHYSICO CHEMICAL CHARACTERIZATION OF SPENT WASH GENERATED DURING ALCOHOL PRODUCTION FROM SWEET SORGHUM JUICE AND ITS IMPACT ON GERMINATION AND SOIL MICRO FLORA

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Abstract: The main objective of this research was to characterize the spent wash generated during alcohol production from sweet sorghum juice(SWSSJ)for its P^H, BOD, COD, TSS and TDS and to study their effect in different concentration on seed germination of selected crops. The SWSSJ was acidic and having BOD and COD value in the range of 22900 mg/l and 35600mg/l respectively. The TSS and TDS of spent wash was found to be 3700mg/l and 8000mg/l respectively. Five different seeds – Soya bean, Black chick pea, White peas, Green gram and Rajma were used to study the effect of different dilution of spent wash in petri plates viz Control without spent wash, 5%, 10%,15%,20%,50% and 100% spent wash. Based on the observation noted in petri dish study, the pot study was done with 2 seeds – White peas and Black chick pea with Control (without spent wash), 5%, 10%,15% and 20% spent wash dilutions. After 15 days of sowing, the soil of different treated pots was studied for number of bacterial and fungal colonies. From the study, it is concluded that the SWSSJ containing high level of plant nutrients can be made available to the plants with proper dilution for the better growth and development of the crop.

Keywords: Sweet sorghum, Characterization, Germination, Soil micro flora

INTRODUCTION

The global urging environmental problem that is associated with the usage of fossil fuel can be mitigated in significant extent by shifting over to usage of renewable fuel resources. (Nigam *et al.*, 2011; Dar *et al.*,2017; Prasad *et al.*, 2018) A noting difference between renewable biofuels and petroleum products is with respect to the oxygen content. Liquid renewable biofuels have chemically bound oxygen levels of 10–45%, whereas petroleum or gasoline has no oxygen rather have hydrocarbons, making the biofuels diverse from those of crude oil with respect to physicochemical properties. (Demirbas2018; Kralova *et al.*,2010) The practice of blending of liquid renewable biofuel ethanol began in India in 2001. The Government of India initiated mandatory biofuel blending programs from 2003 under the National Biofuel Mission. Govt. of India decided to blend 5% ethanol as a fuel additive from November 1, 2006 with a further increase to 10% from October 2008, with efforts to raise it to 20% by the year 2025. (Prasad *et al.*,2012) Currently, the ethanol requirement in India is being fulfilled through fermentation of cane molasses but it is impossible to meet the actual requirement in the long run. (Prasad*et al.*, 2006; Malav*et al.*, 2017) The existing Indian distilleries, consequently, operated at 50% efficiency and needed viable alternative feedstock to perform at their full capacity. (Reddy*et al.*2005) The word "sweet sorghum" is used to describe the cultivar, which contains high amount of sugars in their stalk juice. (Rao *et al.*,2013) World - wide, several high-yielding varieties of sorghum with a fair amount of sugars have been released and are capable of producing biofuel. (Prasad *et al.*,2007; Basavaraj *et al.*,2013) Sweet sorghum is a promising energy crop because it can produce a substantial amount of ethanol from its stalk juice. Furthermore, it has the potential to synthesize soluble sugars (10–20%) in its stalks. (Rao*et al.*, 2010) Its juice contains readily available "total soluble sugars", that can be directly fermented to liquid (ethanol) and gaseous (bio-hydrogen) renewable biofuels. After extraction of sugars from sweet sorghum stalk, i.e., bagasse is available in large amounts that can be used as feedstock in boiler's co-generation process to produce additional energy for sugar mill operations. Also, being an annual crop, it does not occupy the land for a long time unlike other crops like jatropha. Furthermore, cultivating sweet sorghum in dryland conditions does not jeopardize food security as farmers could continue to use grain for foodstuff or feed and stalks juice for renewable biofuels production. (Shiv Prasad *et al.*,2015)

The disposal of wastes from industrial sources is becoming a serious problem throughout the world. The distillery spent wash is acidic and is generally characterized by high levels of biological oxygen demand (BOD) and chemical oxygen demand (COD) and nutrients elements such as nitrogen (N) and potassium (K). Being plant originated, the spent wash also contains considerable amounts of plant nutrients and organic matter. Thus, it can effectively be used as a source of plant nutrients and as soil amendment. The presence of appreciable amounts of plant growth promoters *viz.*,gibberellic acid (GA) and indole acetic acid (IAA)have also been detected

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which further enhances the nutrients value of spent wash. (Murugaragavan 2002)Though, many researches have been carried out to study fermentation of sweet sorghum juice for ethanol production, no sources are available about the characteristics of sweet sorghum based spent wash and its application over Fert-irrigation. The research was designed to study the physio chemical characteristics of SWSSJ and their effect on germination of seeds.

MATERIALS AND METHODS

The present study was carried out at Biochemistry Division, National Sugar Institute, Kanpur**.** Chemicals which have been used in the study of SWSSJ were of AR grade reagents.

Collection of Spent Wash

The sweet sorghum that was harvested from National Sugar Institute (NSI) farm, Kanpur was studied for the ethanol production. The SWSSJ was collected from Distillation unit, NSI, Kanpur for carrying out the current study in a clean, air tight container and properly sealed and stored at room temperature for further analysis.

Analysis of Spent wash

SWSSJ generated was analysed for pH, Biological oxygen demand (BOD), Chemical oxygen demand (COD), Total dissolved solids (TDS) and Total soluble solids (TSS) by standard laboratory methods.

Germination Test in Petri Dishes

The experiment was set up with six dilutions viz 5%, 10%,15%,20%,50%,100% of SWSSJ in duplicates and with a set of control for comparison. The normal tap water was used as control. For germination study in petri plates, 5 different seeds **-** Rajma, Soy bean, Black chick pea, White-Pea and Green gram were used.Uniform seeds were selected, washed and then used. In pre-sterilised petri dishes, Whatman no.1 filter paper was placed and wetted with respective SWSSJ and one set with a control – water. Ten seeds of each variety were sown over each treatment and observed for 5 days. The number of seeds germinated after 5 days was counted for calculation of percentage of germination.

Germination study in Pot

The pot experiment was conducted with 2 varieties of seeds (i.e) Black chick peaand White peas as they gave the best results in petri dish study. Also, the number of treatments was framed on the basis of results obtained as follows **-** 5%, 10%, 15% ,20% SWSSJ and a control with tap water. The soil for the pot study was collected from NSI Farm. Each pot was filled with 1kg dried soil. Before sowing seeds, the soil was made wetted with 1 liter of respective spent wash. Each treated pot was sown with 10 seeds of a variety. The growth was observed for 15 days. During this period, the soil was made wetted every day with their respective percentile spent wash and for control, water was added in the same quantity. After 15 days of seedling, biometric observations were made randomly by selecting one plant on each set. The growth parameters like height of the plant and height of root were measured.

Analysis of Micro flora in Soil Sample

Following proper serial dilution, soil of each treatment was plated for counting number of bacterial colonies and fungal colonies by using Nutrient agar medium and Potato dextrose agar medium respectively.

RESULTS AND DISCUSSION

Analysis of Spent Wash

The SWSSJ collected from Biochemistry lab was analysed for various physical parameters like pH, TSS, TDS, BOD, COD and odour. The results of the analysis are given in the Table 1. The SWSSJ showed 4.95 pH and thuswas acidic in nature. BOD and COD value of SWSSJwere found to be very less compared to the sugarcane based spent wash.

Table 1. General Characteristics of SWSSJ

PARAMETERS	VALUES
pH	4.95
TDS	8000 mg/l
TSS	3700 mg/l
BOD	22900mg/l
COD	35600mg/l
Colour	Brownish yellow
Odour	Unpleasant

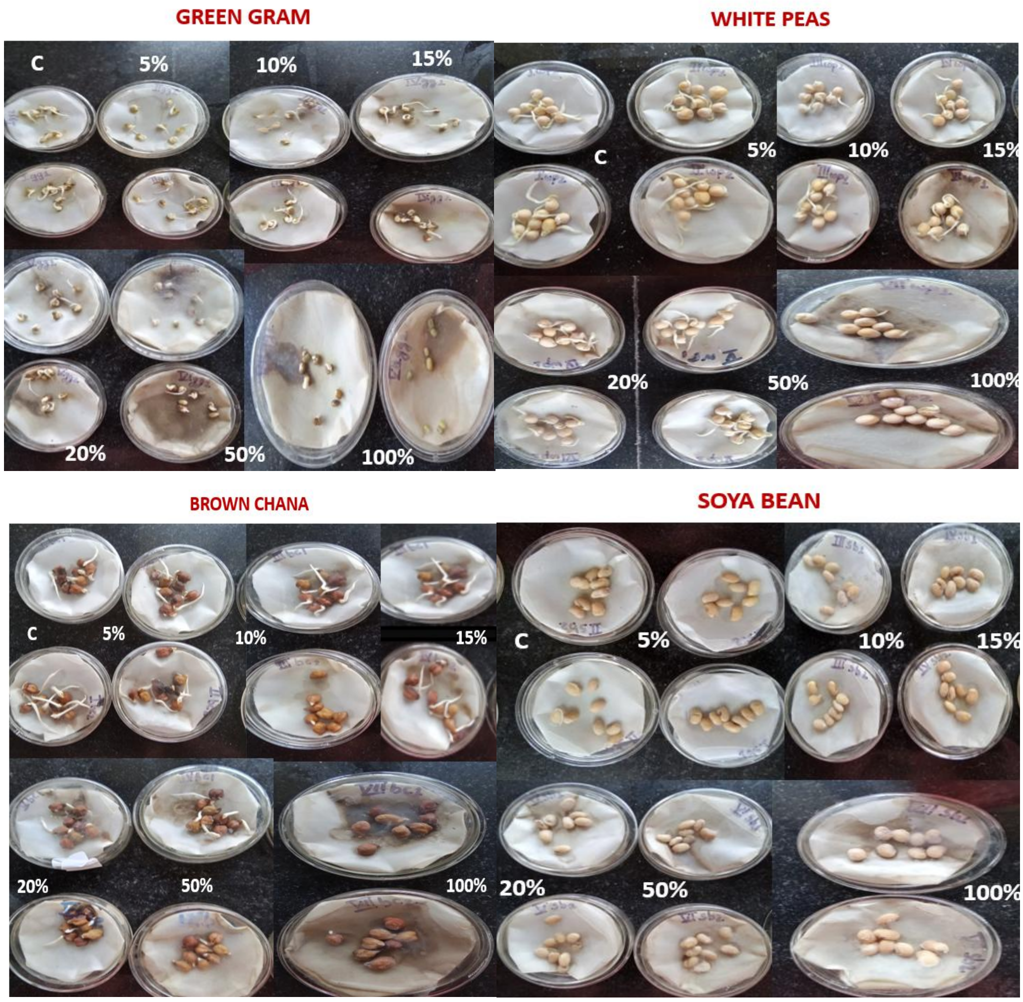
Germination Study in Petri Plates

The germination study results of 5 different seedlings with different diluted proportions of SWSSJ after 5 days of sowing is depicted in the Table 2. The experiment was carried out in duplicates and in each petri plate 10 seeds were kept and observed for germination. Initially 10 ml of treated water was added and thereafter everyday 2 ml of treated water was added in all the treatments till the end of the 5^{th} day of sowing. Then the number of seeds germinated in each set was averaged and noted down. The photos of the same is given in the Fig 1.

The results observed reveals that the five days of germination period is not enough for Soya bean and Rajma as no sprouting was observed in none of the treatments. In White peas, Green gram and Black chick pea, except 100% spent wash treatment, all other treatments showed germination. Though maximum germination was observed in Green gram in control, on fourth day itself the color of the sprouts started to diminish as the life period is short for them in petri plate environment. In all the three varieties that is White peas, Black chick peaand Green gram, in 5% and 15% spent wash treated showed good germination % along with the control and 10% treatment followed them. 20% and 50% spent wash treated one showed less than 50% germination whereas germination was not observed in 100% spent wash treated petri dish of all the varieties.

Table 2. Germination of Seeds in Petri Plates

Treatment	Number of seeds germinated after 5 days/out of 10 seeds sown									
	White peas		Black chick pea		Soya bean		Rajma		Green gram	
	No:	%	No:	%	No:	%	No:	%	No:	%
C	8	80	8	80	0	0	0	0	10	100
5%	6	60	8	80	0	0	0	0	8	80
10%	5	50	5	50	0	0	0	0	7	70
15%	6.5	65	7.5	75	0	0	0	0	8	80
20%	4.5	45	3.5	35	0	0	0	0	4	40
50%	4	40	3	30	0	0	0	0	2	20
100%	0	0	0	0	0	0	0	0	0	0



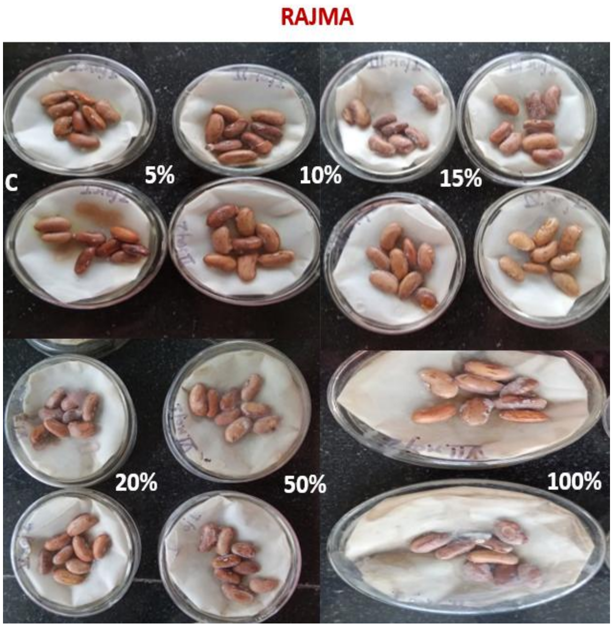


Fig 1. Germination of Seeds at different treatments in Petri dish

Pot Study

The experimental pot study was conducted based on the results obtained in the petri plate study. Only 2 seeds (i.e) Black chick peaand White peas were selected for pot study as best results were observed in those in petri plates. Five treatments were planned as follows – Control, 5% spent wash treated, 10 % spent wash treated, 15% spent wash treated and 20% spent wash treated. Seeds sown in all duplicate treatments were observed for their germination and growth for 15 days. The number of seeds germinated in the treated pots are given in the Table 3.

C – Control

WP – White peas

BC – Black chick pea

Table 3. Germination of Seeds in Pot Study

TREATMENT	**GERMINATION**	
WHITE PEA (WP)	**No:**	**%**
C	10	100
5%	10	100
10%	6	60
15%	9	90
20%	5	50
BLACK CHICK PEA(BC)		
C	7	70
5%	6.5	65
10%	5	50
15%	6	60
20%	4.5	45

From the observed results, it is evident that 5 % SWSSJ treated seeds gave best results more or less as similar as that of control. Followed by that, 15 % spent wash treatment gave best results. The 10 % spent wash treatment as observed in petri dish study, showed less germination % compared to C, 5% and 15% treatments. Finally, 20 % treatment showed only around 50% germination. After 15 days of sowing, the best grown plant among the duplicate was selected and plucked for measuring the Plant stem height and Plant root height from all the treated pots and observed the results as given in the Table 4.

The results revealed that as a whole there is a positive effect of SWSSJ on the growth attributes of 2 seeds studied upto 15th day of observation. In Black chick pea, compared to control, 10 % and 15 % showed marked growth. In 20 % treatment, the growth was in par with control and in 5% the growth of plant was less compared to control. In white peas, compared to control, all spent wash treated showed marked growth except 10% treatment. Though there was a decline in seed germination rate in 15% and 20% treatments, showing good growth attributes in both saplings on these 2 treatments.

Fig 2. Germination of White peas in Pots

Fig 3. Germination of Black chick peain Pot

Table 4. Effect of SWSSJ on Growth Attributes of Black chick pea and White Peas

S. No	Treatments	Black chick pea			White peas		
		Height of stem (cm)	Height of root (cm)	Total Height of the sapling (cm)	Height of stem (cm)	Height of root (cm)	Total Height of the sapling (cm)
1	**C%**	17	3	20	15	1	16
2	**5%**	14	2	16	21	3	24
3	**10%**	20	4	24	13	3	16
4	**15%**	19	3.5	22.5	20.5	2.5	23
5	**20%**	18	2	20	19	1.5	20.5

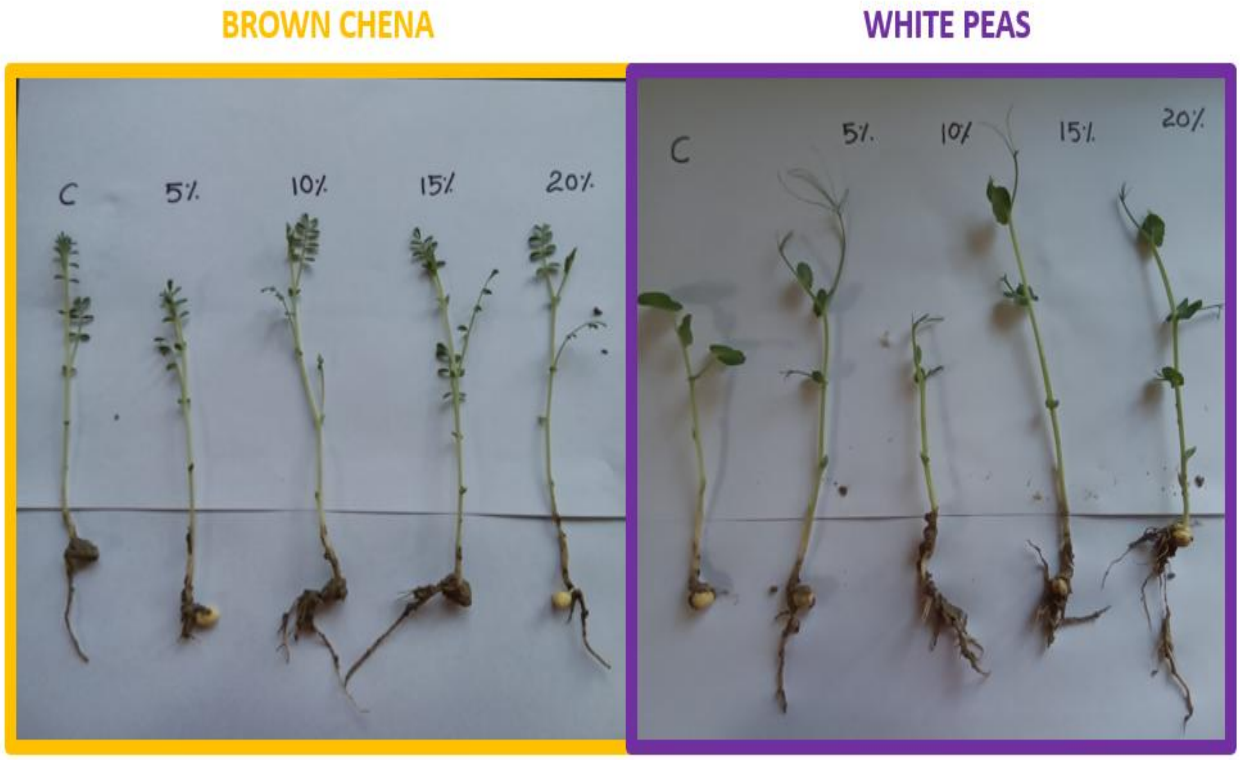


Fig 4. Sapling of Black chick pea and White Peas after 15 days of sowing

Soil Microbial Analysis

The effect of SWSSJ on the soil microbes especially on Bacteria and Fungi was studied and the results obtained are given in the Table 5. From the results it is very clear that increased spent wash concentration has increased the number of microbial colonies in both Black chick peaseeded and White peas seeded soil. The increased growth of soil microbes may be due to the utilization of nutrients present in the spent wash.

Table 5. Soil Microbial Analysis for Black chick pea and White Peas

S.No	Treatment	Black chick pea		White Peas	
		Bacteria (CFU/ml)	Fungi (CFU/ml)	Bacteria (CFU/ml)	Fungi (CFU/ml)
1	C%	$6.8 X 10^5$	$9.4 X 10^5$	$2.1 X 10^5$	$5.4 X 10^5$
2	5%	$7.1 X 10^5$	$12.3 X 10^5$	$3.5 X 10^5$	$9.2 X 10^5$
3	10%	$8.0 X 10^5$	$12.4 X 10^5$	$6.0 X 10^5$	$11.3 X 10^5$
4	15%	$9.4X 10^5$	$15.1X 10^5$	$10.5X 10^5$	$11.5X 10^5$
5	20%	$14.5 X 10^5$	$17.5 X 10^5$	$11.7 X 10^5$	$18.3 X 10^5$

The treatment of molasses-based distillery spent wash at higher concentration with soil promotes fungal growth over the soil and inhibits the seed germination. This should be considered for prolonged application at higher concentration.

CONCLUSION

The emerging need of ethanol production for Ethanol Blending Program has forced us to look for an alternate feed stock for ethanol production. The expansion of distilleries has already posed severe shortage of molasses, therefore to keep the distilleries functional, the launch of flexi feed policies has turned into the need of the hour. In order to cope up with 20% EBP and make it success, planning for utilization of each alternative feed stock become a need, so that the states where sugar cane is not grown other feed stock plants like sugar beet, maize, cassava, sweet sorghum can be grown or in the same distilleries additional pre-treatment portion can be added and flexi feed can be done. Studying the properties and characteristics of spent wash generated during ethanol distillation and its utilization is also important as that of the production of the ethanol. From the study, it is concluded that the SWSSJ containing high level of plant nutrients can be made available to the plants and that result in better growth and development of the crop. There may be a declining tendency of growth parameters in higher concentration due to the presence of higher amount of organic matter, BOD which leads to depletion of O_2 and accumulation of CO_2 in the soil. It is recommended based on the current study that sweet sorghum distillery spent wash up to 20 % concentration can be applied for the cultivation of crops.

SUGGESTION FOR FURTHER STUDIES

SWSSJ should be further analysed for the presence of heavy metals and other components. Also, while utilizing the spent wash for agriculture purpose, the CPCB norms should be abided. The pot study should be observed for entire harvesting period. The yield characteristics of spent wash treated should be studied. Field application studies can be initiated after proper dilution and the same can be tried with other crops.

Author Contributions

All authors contributed to the study conception and design. All authors read and approved the final manuscript.

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