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Effect of Earthworm Castings on Ethanol Production by *Saccharomyces cerevisiae*

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ABSTRACT

With increase in population and industrialization the demand of energy is increasing day by day. Simultaneously, the worldwide demand of ethanol as biofuel is increasing constantly. Ethanol can be produced by using major traditional agricultural crops e.g. sugarcane, maize and sugar beets but these crops are not able to meet the global demand of ethanol as these crops possess primary value of food and feed. Deteriorated sugar, an agro-residue, could be an attractive feedstock for production of ethanol. Earthworm (*Eisenia fetida*) cast a rich source of nitrogen and phosphorus, may be considered as alternative of chemical fertilizers required for the growth of yeast during fermentation of deteriorated sugar. In present study, sustainable ethanol production from deteriorated sugar is investigated with the use of earthworm cast. On fermentation of 14% deteriorated sugar supplemented with 0.5% earthworm cast 5.3% ethanol production was observed after 72 h incubation at 30±2ºC that was higher as compared to control without supplementation with cast.

Keywords Deteriorated sugar, *Eisenia fetida*, Ethanol, Fermentation, *Saccharomyces cerevisiae*.

INTRODUCTION

Ethanol from renewable resources has been of interest in recent decades as an alternative fuel or oxygenate additive to the current fossil fuels (Parsad *et al.* 2007,Gashaw and Teshita 2017). Conventional sources of sugars viz., sugarcane molasses, grains and sugarbeet cannot meet the increasing demand of ethanol due to food vs fuel debate (Nigam and Singh 2011, Shivasakthivelan *et al.* 2014). Alternate substrates for ethanol production are explored by various workers. Rani *et al.* (2010) explored ethanol production from spoiled potato *(Solanum tuberosum)* flour and observed 56.8 g/L ethanol after 48 h fermentation at 30ºC while Goel and Wati (2013) reported 20.46 g/L ethanol production from rice *(Oryza sativa)* straw biomass. Deteriorated sugar is one of the abundant waste materials of sugar mills in the world (Eggleston and Lima 2015). Chemical fertilizers like disodium hydrogen phosphate, urea and ammonium sulfate or ammonium phosphate are generally used as nutrients in the fermentation of molasses and other sugars because yeast cannot grow and ferment well without minerals like nitrogen and phosphorus (Thippareddy and Agrawal *et al.* 2010). Earthworm

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Fig. 1. Earthworm cast.

cast, a rich source of nitrogen and phosphorus, is also very cheap, easily available and least toxic mineral source. Deteriorated sugar supplemented with earthworm cast can be used for ethanol production which would be a renewable eco-friendly process (Chen *et al.* 2007). In present investigation the application of *Eisenia fetida* castings as supplement to deteriorated sugar for the production of fuel alcohol was explored under laboratory conditions.

Fig. 2. Deteriorated sugar.

Fig. 3. *Saccharomyces cerevisiae (*HAU-1) strain.

MATERIALS AND METHODS

Earthworm cast: Cast of *Eisenia fetida* was obtained from vermicomposting unit, Department of Zoology, CCS Haryana Agricultural University, Hisar, Haryana. The cast (Fig. 1) was analyzed for total Nitrogen, Phosphorus, Potassium and Organic Carbon as per standard methods (AOAC 1990).

Deteriorated sugar: Deteriorated cane sugar for present study was obtained from sugar mill, Meham (Rohtak) (Fig. 2).

Yeast culture: A fast fermenting strain of *Saccharomyces cerevisiae* (HAU-1) (Fig. 3) in the present study was procured from culture collection, Department of Microbiology, CCS Haryana Agricultural University, Hisar, Haryana. The culture was maintained on yeast extract peptone dextrose (YEPD) medium containing Yeast Extract 10 g/L, Peptone 20 g/L, Dextrose 20 g/L slants by regular sub-culturing and stored at 4±1°C until further use.

Inoculum medium (IM): For inoculum preparation, Yeast Extract Peptone Sucrose (YEPS) medium containing Yeast Extract 5 g/L, Peptone 5 g/L, Sucrose 60 g/L was used. One hundred ml inoculum medium was pitched with 24 h old culture of *Saccharomyces*

Fig. 4. Ethanol production from deteriorated sugar supplemented with earthworm cast.

cerevisiae and the flasks were incubated for 24 h at 30±1ºC under shaking conditions.

Fermentation: To study the effect of vermicast of *E. fetida* on ethanol production, 14% deteriorated sugar solution was mixed with different concentrations (0 – 5% w/v) of vermicast. The solution was sterilized at 121°C for 15 min. Twenty four hour old yeast inoculum was added to this sterilized solution and the contents were fermented for different time intervals at 30±2°C in BOD incubator (Fig. 4).

Ethanol estimation: Ethan content in fermented broth was estimated spectrophotometrically according to the method described by Caputi *et al.* (1968).
Statistical analysis : The experimental data was analyzed, using 'OPSTAT' software, available on CCS Haryana Agricultural University, Hisar website www.hau.ac.in.

RESULTS AND DISCUSSION

For sustainable ethanol production to be used as bio-fuel, deteriorated sugar, a waste of sugar mills was fermented using earthworm cast as N and P source. The composition of earthworm cast (as presented in (Table 1) showed significant amount of N and P suggesting suitable supplement for ethanol fermentation using deteriorated sugar. High concentration of N and P in earthworm cast has also been reported by various workers. Hadis *et al.*(2018) reported 11.37% organic carbon, 1.41% N, 0.78% P, 1.02% K in *Eisenia fetida* cast while Mistry *et al.* (2015) reported 1.7% N, 1.3% P, 0.8% K and 30% organic carbon in Perionyxexcavatus cast.

Table 1. Composition of earthworm *(Eiseniafetida)* cast.

Component	Value (% DW basis)
Organic carbon	33.70
Nitrogen	1.75
Phosphorus	0.76
Potassium	0.92
C:N ratio	19:27

Table 2 . Ethanol production from deteriorated sugar and different concentrations of earthworm cast using *Saccharomyces cerevisiae.*

Earthworm cast (% w/v)	Ethanol content (v/v)
0.3% Urea + 0.15% Disodium hydrogen phosphate (Control)	5.48
0	0.83
0.25	4.57
0.50	5.30
1.00	5.03
2.00	4.98
5.00	4.88
CD (p=0.05)	0.018
SE (m)	± 0.006

The results on ethanol production from deteriorated sugar using different concentrations of earthworm cast presented in Table 2 and Fig. 5. Ethanol production increased with increasing supplementation of earthworm cast in deteriorated sugar. Maximum i.e. 5.3% ethanol production after 72 h was observed at 0.5% supplementation of cast that decreased with further increase in concentration of cast. While on supplementing urea (0.3%) and disodium hydrogen

Table 3. Ethanol production from deteriorated sugar supplemented with 0.5% earthworm cast at different time intervals cast using *Saccharomyces cerevisiae.*

Time (h)	Ethanol (v/v)
0	0.83
24	2.13
48	3.00
72	5.30
96	5.00
CD (p=0.05)	0.073

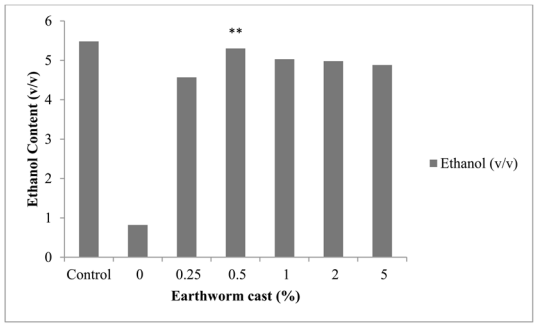

Fig. 5. Effect of different concentrations of earthworm cast by *Saccharomyces cerevisiae*** p< 0.05 versus 0 % earthworm cast.

phosphate (0.15%) in 14% deteriorated sugar solution 5.48% ethanol content was observed. Gupta *et al.*(2012) reported 0.9% increase in ethanol yield when supplemented with 14% deteriorated sugar supplemented with 0.5% *Pheretima posthuma* excreta. They proved that earthworm cast is fully capable in producing significant amount of ethanol when supplemented with deteriorated sugar in the presence of yeast.

The maximum ethanol content (5.3%) was observed at 0.5% of earthworm cast as nitrogen and phosphorus source in deteriorated sugar solution. Yeast strains differ in their fermentation efficiency and productivity (Mussatto *et al.* 2012). The effect of incubation time on ethanol fermentation was determined by fermenting 14% deteriorated sugar supplemented with 0.5% earthworm cast. The ethanol content was found to increase upto 72 h (5.3%) and decreased thereafter (Table 3, Fig. 6). Similarly Snehlata and Wati (2019) reported maximum ethanol content in combined fermentation of different waste (paddy straw and cheese whey) by *Saccharomyces cerevisiae* (HAU-1) after 72 h of fermentation at 35ºC. Wati and Goel(2020) reported maximum ethanol i.e.31 ml/L with the combined fermentation of paddy straw and cheese whey by *Saccharomyces cerevisiae* (HAU-1) after 72 h.

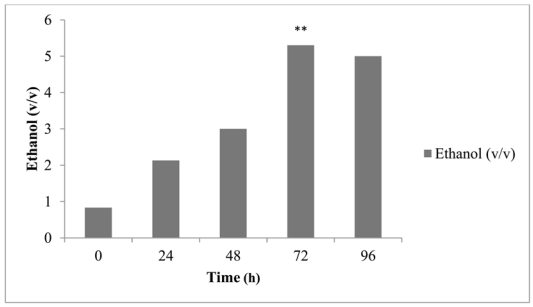

Fig. 6. Effect of incubation time on ethanol production from deteriorated sugar supplemented with 0.5% earthworm cast by *Saccharomyces cerevisiae*** p< 0.05 versus 0 h.

CONCLUSION

Earthworm cast a rich source of nitrogen and phosphorus, may be considered as alternative of chemical fertilizers required for the growth of yeast during fermentation and for ethanol production replacing urea and disodium hydrogen phosphate which are very costly and toxic as well.

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