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Solid-State Fermentation from Dried Sweet Sorghum Stalk for Bioethanol Production

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Abstract: Due to depletion of global crude oil, countries are interested to alternate fuel energy resources. Presently bioethanol as a source of energy has been a subject of great interest for the industrialized countries. Therefore, there is a need for efficient bioethanol production with low cost raw material and production process. Among energy crops, sweet sorghum is the best candidate for bioethanol production. It has been identified as having higher drought tolerance, lower input cost and higher biomass yield than other energy crops. In addition it has a wide adaptability and tolerance to abiotic stresses. Water requirement of sweet sorghum is one third of sugarcane and one half of corn that are currently used for bioethanol production. Moreover due to the shortage of water in dry and hot countries, there is a need to reduce water requirements for bioethanol production and solid state fermentation could be the best process for making bioethanol in these countries. The purpose of this study was to achieve the highest ethanol yield with lowest amount of water in solid state fermentation using dry sweet sorghum stalks. Fermentation medium were: dry sweet sorghum powder with nutrient media, active yeast powder and different moisture contents. The fermentation medium was incubated for 48 to 96 hr.at 35 ºC temperature. The results showed sweet sorghum powder (15% w/w) fermented in medium containing 0.5% yeast inoculums, 60% moisture content and 72 hr. incubation period produced the highest ethanol yield of 12.16% w/w dry sweet sorghum powder.

Keywords: Sweet sorghum, Bioethanol, Solid state fermentation, Water content, Biomass,

1. Introduction

With growing concerns for energy security, environmental pollution and future oil supplies, countries are seeking renewable energy (Ergum, 2000). Among renewable energy bioethanol as a transportation fuel, has a great potential for energy and clean environment. Presently, the main feed stocks for bioethanol production are corn, wheat, sugarcane and sugar beet. These are consumed by human and animals; therefore, there is a need to find another feedstock suitable for making ethanol. Sorghum bicolor (L.) Moench is a C4 crop characterized by high photosynthetic efficiency, drought and salinity resistance (Ali et al., 2011; Demirbas, 2008; Almodares and Hadi, 2009; Vasilakoglou et al., 2011). Sweet sorghum is well adapted to the dry and hot climatic conditions and therefore it is one of the most promising crops for ethanol production under these climatic conditions (Shen and Liu, 2009). Due to its high yielding and sugar content, it has been considered as an energy plant for ethanol production (Kwon et al., 2011). Generally mills are used to extract the juice from sweet sorghum stalks. The sugars in fresh stalk or juice will be easily deteriorated in ordinary condition and the harvest season from sweet sorghum in most countries is limited from September to December, resulting in shortage of raw materials for whole year. To overcome the above two problems, it is necessary and important to dry sweet sorghum stalks for ethanol production. In this way total sugar and reducing sugar could be unchanged for almost a year (Gnansounoua et al., 2005). This could be due to low water activity and higher sugar concentration that were not suitable for the growth of many microorganisms (Bryan, 1990).

Solid-state fermentation has gained renewed attention, not only from researchers but also from industries, due to several advantages over submerged fermentations. This is partly because solid-state fermentation has lower energy requirements, higher yields, produces less wastewater with less risk of bacterial contamination, and partly because of

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environmental concerns regarding the disposal of solid wastes (Ali et al., 2011).
Solid-state fermentations (SSF) are fermentations of solid substrates at low moisture levels or water activities; however, the substrate must possess enough moisture to support growth and metabolism of the microorganism. The water content of a typical submerged fermentation (SmF) is more than 95 %, while water content of a solid mash in solid-state fermentation often varies between 40 % and 80 %.

Ethanol production from sweet sorghum dry stalk particles using solid state fermentation with different water contents was tested (Kwon et al., 2011). It was reported the suitable water content for ethanol production and yeast growth was 76.47%.

Solid state fermentation of chopped sweet sorghum particles to produce ethanol was studied statically using thermotolerant yeast (Yu et al., 2008). They found that maximum ethanol yield (0.46 g ethanol/g total sugar) was obtained with 4×10^6 yeast cell concentration/ g raw sorghum, 1.5 mm particle size and 75% moisture content.

The highest ethanol yield of 0.25 g-ethanol/g-dry sweet sorghum stalk was obtained at 37°C with 15-20 cm substrate depth in the bioreactor. These results provided a great potential for large-scale deep-bed SSF in practice (Kwon et al., 2011). In another study 0.298 g ethanol/g dry sweet sorghum stalks was obtained (Song et al., 2007).
Iran has a dry and hot climatic condition. The average rainfall is less than 250 mm per year. So in order to produce bioethanol as a transportation fuel, sweet sorghum is well adapted to these conditions. Water consumption for ethanol production is 5 gallons water/gallon fuel ethanol (Keeney and Muller, 2006). Therefore solid state fermentation of sweet sorghum is the most promising procedure for biofuel production under Iranian hot and dry climatic condition. The purpose of this study was to optimize moisture content, sweet sorghum dry powder, amount of yeast and incubation period on ethanol yield.

2. Materials and Methods

The experiment was carried out at the University of Isfahan Research Station. In May 2011 sweet sorghum seeds var. Sofra were planted and the plants were harvested at physiological maturity. The panicles and leaves were stripped from fresh stalk. The fresh stalks were chopped and ground into wet particles with corn chopper for less than 4 mm length. The wet sweet sorghum stalk particles were sun-dried. The dried particles were stored without being sieved Storage of sweet sorghum stalk in dry particles would be long-time, effective with low energy consumption. The dry particles were ground to 60 mesh powders by a mill before starting the experiment. For preparation of salt buffers, 8-30 g sorghum particles suspended in 30 ml salt buffer containing: 0.3 g $(NH_4)_2SO_4$, 0.15 g KH_2PO_4 , 0.06 g $MgSO_4$, $7H_2O$. For each treatment, sterilization vs. not sterilization was tested. Sweet sorghum particles and the buffer autoclaved at 121°C for 15 minutes then cooled to room temperature for yeast inoculation. For preparation of yeast inoculum (*Saccharomyces cerevisiae*), 0.05-1.0 g from yeast powder suspended in 5 ml sterile distilled water incubated 5 min at 37 C and 20 min at 30 C for reactivation of microorganism. This activated yeast inoculated to medium containing sorghum particles in flask and mixed well, then sealed by parafilm for prevention of entering air into flask. Ethanol fermentation proceeded at 35^0 C for 48,72 and 96hr. Ethanol concentration was determined by colorimetric assay using potassium dichromate and sulfuric acid method according to (Ergum, 2000). One way analysis of variation (ANOVA) was carried out to compare the means of results at different runs. The significant F values were obtained and the Duncan's Multiple Range Test was applied to determine the significant differences among the different treatments.

3. Results and Discussion

3.1 Optimization of sterilization and moisture content for ethanol production

Sterilization, moisture contents and their interactions were significant ($P<0.01$). Mean comparison for sterilization and moisture contents are shown in Table1. Sterilization had significantly higher ethanol yield (11.05 percent) than not sterilization (11.89 percent) which indicate may be other microorganisms interfere with the yeast. There was no significant difference between 60 percent, 70 percent and 80 percent moisture contents for ethanol yield and on average was 11.81 percent. As the amount of moisture content increased, ethanol yield was reduced significantly. This could be due to diluting the medium and sugar concentration.

Interaction between sterilization vs. not sterilization and moisture contents on ethanol yield in fermentation medium with 15 g dry sorghum powder and 0.5 gr yeast incubated for 3 days is shown in Fig 1.The highest amount of ethanol yield (12.16 percent) was obtained with 60 percent moisture content and sterilization. The lowest amount of ethanol yield (9.73 percent) was obtained with 120 percent moisture and not sterilization. As the amount of moisture content increased, ethanol yield decreased significantly. This reduction was higher in not sterilization than sterilization. Moisture content and sterilization effect considerably on ethanol yield. Sugar dilution and other microorganisms in not sterilization could effect on ethanol yield.

3.2 Optimization of sterilization and dry sweet sorghum powders on ethanol yield

Sterilization, amount of sweet sorghum powder and their interactions were significant ($P<0.01$). Mean comparison for sterilization vs. not sterilization and moisture content are shown in Table 2.

Highest amount of ethanol yield was 11.64 percent with 15 g dry sweet sorghum powder. Higher and lower amounts (8 g or 30 g) reduced ethanol yield significantly. It was 11.37 percent with 8 g sweet sorghum powder and 10.74 percent with 30 g sweet sorghum powder. It seems that there were not enough feedstock (8 g) for the microorganism to produce ethanol yield and too much feedstock (30 g) which reduce the activity of the microorganism. Similar to moisture content, ethanol yield was significantly higher in sterilization (11.15 percent) than not sterilization (11.05 percent).

The interaction between sterilization vs. not sterilization and sweet sorghum powder on ethanol yield in fermentation medium with 60 ml moisture and 0.5 g yeast incubated for 3 days is shown in Fig.2. The highest amount of ethanol yield was obtained with 15g dry sweet sorghum power and sterilization (11.80 percent) and lowest was (10.62 percent) with 35 g dry sweet sorghum powder and not sterilization). Higher or lower than 15 g dry sweet sorghum powder reduced ethanol yield significantly.

3.3. Optimization of sterilization and yeast for ethanol yield

Amount of yeast and the interactions of sterilization and amount of yeast were significant ($P<0.01$) for 60 ml moisture, 15.0g sweet sorghum powder incubated for 3 days. Mean comparison for sterilization vs. not sterilization and yeast content are shown in Table3. 0.5g and 0.8 g yeast produced the highest ethanol yield (11.26 percent on average). Ethanol yield was 10.56 percent which lower than both 0.5g and 0.8g yeast. As the amount of yeast decreased below 0.5 g namely 0.2g, 0.1g and 0.05g, ethanol yield was reduced significantly. Under low amount of yeast, there is not much yeast to convert sugar in the dry sweet powder to be converted to ethanol.

The interaction between sterilization vs. not sterilization and amount of yeast on ethanol yield in fermentation medium with 60 ml moisture and 0.5 g yeast incubated for 72 hr is shown in Fig.3.

Since sterilization vs. not sterilization was significant, therefore the trend of ethanol production was similar according to Table 4. However in each amount of yeast, the ethanol yield was higher in sterilization than not sterilization, although the differences were not significant.

3.5. Optimization of sterilization and incubation period

Amount of yeast and their interactions with sterilization were significant ($P<0.01$). Mean comparison for incubation period is shown in Table4. Highest ethanol yield (11.75 percent) was obtained with 72 hours incubation period. Ethanol yield significantly decreased in less than 72 hours (9.05 percent ethanol in 48 hours) and 9.78 percent ethanol in 96 hours). It seems 48 hours incubation period is not enough for the yeast to convert sugar to ethanol. On the other hand 96 hours incubation period caused yeast used the sugar for its growth rather than producing ethanol.

However retention time was less than 30hr, using sweet sorghum stems with advanced solid state fermentation technology in continuous solid- stage fermenter (55 m in length and 6.3 m in diameter (lie et al., 2013).

The interaction between sterilization and incubation period is present in Fig4. Even the interaction between sterilization and incubation period was not significant, but the highest and lowest amount of ethanol was obtained with 72 hours incubation (11.86 percent) 48 hours of incubation (9.19 percent), respectively. Ethanol yield was not significantly different for sterilization and not sterilization, although ethanol yield was higher for sterilization than not sterilization.

4. Conclusions

Due to depletion of global crude oil, countries are interested to alternate fuel energy resources. Presently bioethanol as a source of energy has been a subject of great interest for the industrialized countries. Among energy crops, sweet sorghum is the best candidate for bioethanol production. It has been identified as having higher drought tolerance, lower input cost and higher biomass yield than other energy crops. Moreover due to the shortage of water in dry and hot countries, there is a need to reduce water requirement for bioethanol production and solid state fermentation could be the best process for making bioethanol in these countries. In this study solid state fermentation of different dry sweet sorghum stalk, amount of water and incubated days was investigated. The results showed sweet sorghum powder (15% w/w) fermented in medium containing 0.5% yeast inoculums, 60% moisture content and 72hr incubation period produced the highest ethanol yield of 12.16% w/w dry sweet sorghum powder.

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Table1: Effect of sterilization vs. not sterilization and moisture contents on ethanol yield.

Treatment	Ethanol yield (%)	Moisture content (ml)	Ethanol yield (%)
Sterilization	11.05a*	60	11.86a*
Not sterilization	10.89b	70	11.76a
		80	11.81a
		90	11.00b
		100	10.60c
		110	10.32d
		120	9.43e

* Value followed by the same letter in a column is not significantly different at 5% level using Duncan Multiple Range Test.
Fermentation condition: 15 gr dry sweet sorghum powder, 0.5 g yeast, pH 5, 35^{0}C incubated for 3 days.

Table2: Effect of sterilization vs. not sterilization and dry sweet sorghum powder on ethanol yield.

Treatment	Ethanol yield (%)	Sweet sorghum powder (g)	Ethanol yield (%)
Sterilization	11.15a*	8	11.37b*
Not sterilization	11.05b	15	11.64a
		30	10.74c

* Value followed by the same letter in a column is not significantly different at 5% level using Duncan Multiple Range Test.
Fermentation condition: 60 ml moisture, 0.5 g yeast, pH 5, 35^{0}C incubated for72hr.

Table3: Effect of sterilization and yeast on ethanol production

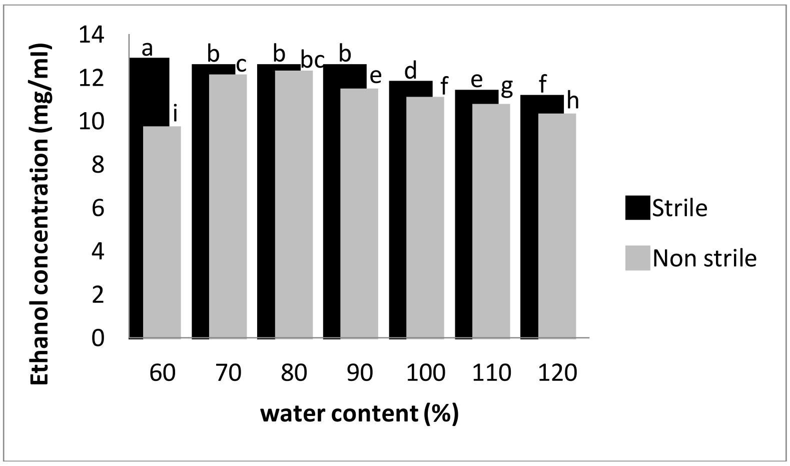
Treatment	Ethanol yield (%)	yeast (g)	Ethanol yield (%)
Sterilization	9.21a*	1	10.56b*
Not sterilization	9.08a	0.8	11.33 a
		0.5	11.18 a
		0.2	8.58c
		0.1	7.62d
		0.05	5.91e

* Value followed by the same letter in a column is not significantly different at 5% level using Duncan Multiple Range Test.
Fermentation condition: 60 ml moisture, 15.0g sweet sorghum powder, pH 5, 35^0C incubated for72hr.

Table4: Effect of sterilization and incubation period on ethanol production

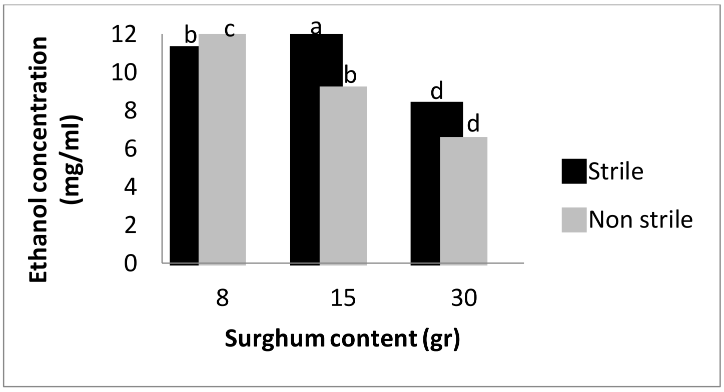
Treatment	Ethanol yield (%)	Incubation period (hr.)	Ethanol yield (%)
Sterilization	10.30a*	48	9.05c*
Not sterilization	10.09a	72	11.75a
		96	9.78

* Value followed by the same letter in a column is not significantly different at 5% level using Duncan Multiple Range Test.
Fermentation condition: 60 ml moisture, 15.0g sweet sorghum powder, 0.5 g yeast, pH 5, 35^0C



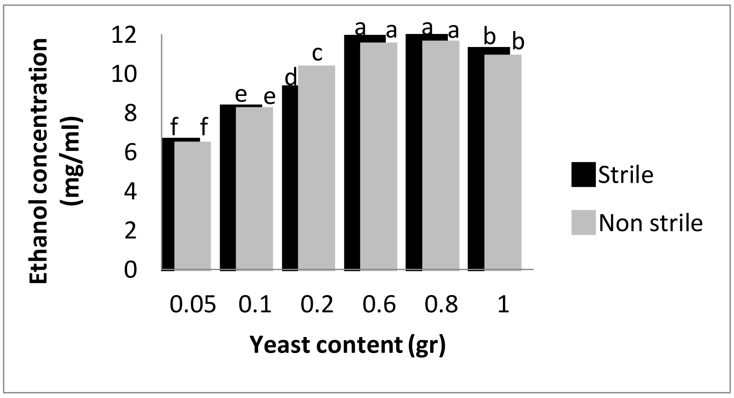
Value followed by the same letter is not significantly different at 5% level using Duncan Multiple Range Test.

Fig.1: Effect of sterilization and moisture content on ethanol yield in fermentation medium with 15 g sorghum and 0.5g yeast incubated for 72 hr.



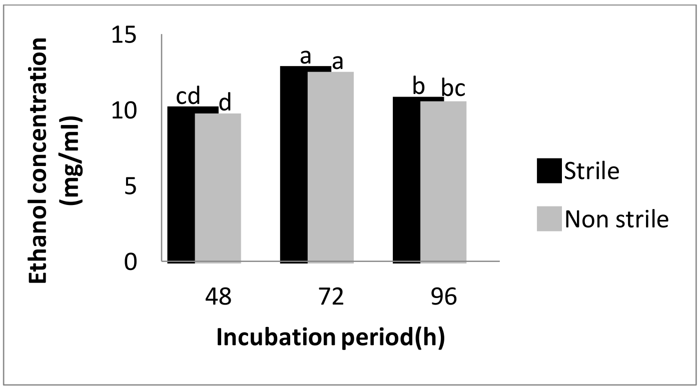
Value followed by the same letter is not significantly different at 5% level using Duncan Multiple Range Test.

Fig. 2: Effect sterilization and sorghum powder on ethanol yield in fermentation medium with 60 ml moisture content, 0.5 g yeast incubated for 72hr.



* Value followed by the same letter in a column is not significantly different at 5% level using Duncan Multiple Range Test.

Fig.3: Effect of yeast inoculation content on ethanol production in fermentation medium contain 60% water and 15 gr sorghum powder incubated 72 hr.



* Value followed by the same letter in a column is not significantly different at 5% level using Duncan Multiple Range Test.

Fig.4: The effect of incubation periods on ethanol production in fermentation medium containing 15.0 gr. dried sorghum powder, 60 ml moisture , 0.5 gr dried yeast, pH 5, 35^{0}C.