Plant Archives Vol. 19, Supplement 2, 2019 pp. 345-350 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

FURFURAL PRODUCTION BASED CELLULOSIC GARBAGE

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Abstract

Fermentative produced ethanol from lignocellulose by fungi is a renewable alternative to fossil fuels. However, the breakdown process of the raw material by the fermentation made known the furan compounds furfural. In the current work, the furfural this was done using *in vitro* spectro-photometric assays. The kinetic analysis suggested that furfural formed by fungi metabolism. For those reasons the study was aimed the following: Furfural products from cellulosic garbage, and what is the best PH, time and sample type to its accumulation.

Keywords : Cellulosic garbage, lignocellulose, fungi metabolism.

Introduction

The current extensive use of fossil resources for energy production is well known to significantly influence the environment. In order to lower the impact on the environment, and find an economical alternative to fossil fuels, a changeover to energy sources made from renewable resources is an attractive strategy. One renewable resource is lignocellulose, which is also widely available, Ethanol can be fermentative produced and utilized as an alternative energy source (Hakan and Greaker, 2010).The Conversion of garbage materials to fuels which use abundant non-edible cellulosic biomass as feedstock (Cherubini and Ulgiati, 2014). However, lignocellulosic biomass is highly recalcitrant due to its complex composition of cellulose, hemicellulose, and lignin (Chundawat *et al*., 2016). The production of ethanol from lignocellulose requires pretreatment steps preceding the fermentation, in which the sugar polymers are made accessible to the fermenting microorganism (Modig, 2003). Fuel molecules are include ethanol, butanol, isobutanol, biofuel, bisbolene, organic acids, long-chain alcohols (Balan *et al*., 2013), and furfural (Ratledge and Wynn, 2002). The biofuel produced by oleaginous organisms are. Furfural is attractive feedstocks for production of renewable fuels due to its high carbonto-heteroatom ratios (Knothe, 2010). Oleaginous microbes An 'oleaginous' microorganism is one that is able to accumulate greater than 20% of its dry body mass as Fuel molecules (Ratledge and Wynn, 2002; Ratledge,2011). A literature survey by Subramanian *et al*. included: microalgae, yeast and molds, the yeast *Rhodosporidium toruloides*, *Cryptococcus curvatus*, *Candida sp.* the bacterium *Rhodococcus opacus*, the mold *Mortierella ramanniana*, the yeast *Trichosporon cutaneum*, *Trichosporon fermentans*, and the mold *Cunninghamella echinulata*. Currently, only a few techno-economic studies have been conducted on the use of oleaginous microorganisms for the production of SCO (Gong *et al*., 2014). *Candida curvata* (Ratledge and Cohen, 2008).

Furfural advantages

A span of about 100 years marked the period from discovery of furfural in the laboratory to the first commercial production in 1922, The subsequent industrial development provides an excellent example of the industrial utilization of agricultural residues (Britannica, 2018). Furfural was first isolated in 1821 the German chemist Johann Wolfgang Döbereiner, who produced a small sample as a byproduct of formic acid synthesis At the time, formic acid was formed by the distillation of dead ants. In 1840, the Scottish chemist John Stenhouse found that the same chemical could be produced by distilling a wide variety of crop materials, including corn, oats, bran, and sawdust, with aqueous sulfuric acid; he also determined an ($C_5H_4O_2$) Furfural is an organic compound derived from variety of agricultural by-products. The name furfural comes from the Latin word furfur, meaning bran, referring to its usual source. based, chemical feedstock Furfural is an important renewable, non-petroleum furfural is an aldehyde of furan. It is a colorless oily liquid with the odor of almonds, which quickly darkens when exposed to air. The first of these obstacles was relieved when it was found that carbohydrate and cellulosic agricultural wastes could be cheaply and efficiently converted to furfural and Synthesis of amino acids from biomass based 5-hydroxymethyl furfural (Taherzadeh *et al.,* 2000). Furfural and HMF usually coexist during bioethanol production, and reportedly can act synergistically to suppress yeast cell growth even at low concentrations (Santacesaria *et al*., 2012). precursor to furan-based chemicals, petroleum refining, Precursor for wide range of chemical syntheses, Chemical solvent extraction , Insecticide and Herbicides, thermoset polymer matrix, Fiber glass, the plastic matrix, Adhesives and Epoxy, Lubricant, casting resins, coatings and floor grouting, food flavorings and organoleptic, Biofuels and fuel products, pharmaceuticals, Antimicrobial agents, Antibiotic, Detergents and Cosmetics, Fertilizers industry, cleaning electronic component, Rubber industry, foundry cores and molds (Adil, 2002; Iwaki, 2013).

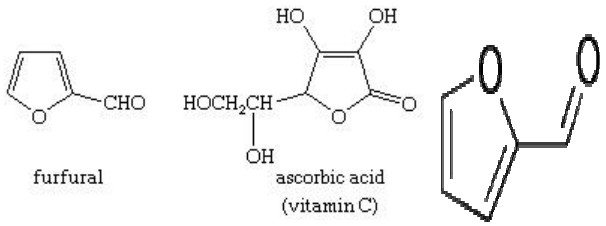


Fig. 1: Furfural structure

Pretreatment of Garbage

A key step, involves the application of physical, chemical, thermal, or biological forces to disrupt the carbohydrate complex in the cell walls of plants in cellulosic biomass and to increase the sugar yield of enzymatic hydrolysis (Yang

and Wyman, 2008; Balan, 2014). Some of the leading pretreatment technologies that have been successfully include Dilute Acid (DA) or Steam Explosion to hydrolyze the major polysaccharides (cellulose and hemicellulose) into simple sugars (Van Dyk, and Pletschke, 2012). Since the sugars derived from cellulosic biomass are mostly glucose and xylose, fungi that consume both glucose and xylose from celluloses are desirable (Huang, *et al.,* 2013). However, solid-state fermentation has drawbacks in areas such as heat and mass transfer, scale-up the cell and chemicals harvest. The fuel content of cell biomass generated during fermentation of cellulosic hydrolysates was lower overall than that seen in synthetic media. For instance, (Xie *et al.*, 2012). Lipid fermentation is generally a slower process compared with ethanol fermentation (Tsigie *et al*., 2012).
Pretreatment is a combination of many processes. It consists of a size reduction step as shown in Figure (1) lignocellulose is mainly made up of lignin, hemicellulose, and cellulose fibers. These all combine to form a firm, compact network structure. In a natural state, after size reduction, the access to cellulose is still blocked by lignin and hemicellulose because of the intact cell wall structure. Moreover, cellulose has a highly crystalline structure that is difficult to break down (Liu *et al.*, 2012). Because pretreatment is the first step of the biofuel process for ligno-cellulosic biomass, the quality and efficiency of pretreatment directly affect the subsequent steps, including enzyme hydrolysis and fermentation steps.

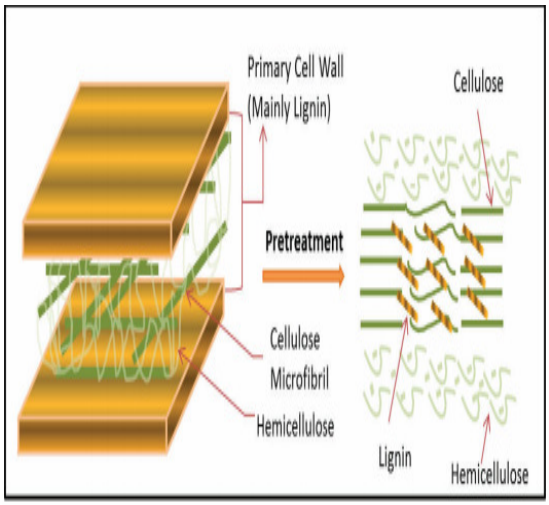


Fig. 2: lignocellulose is mainly made up of lignin, hemicellulose, and cellulose fibers

Furfural Recovery

The fermentation broth media involves harvesting cells from the broth, either by drying cell biomass or by forcing cell disruption, and furfural compounds extraction. Cell harvest is expensive when cell density is low in the fermentation broth. Commonly used cell-harvesting methods include centrifugation, filtration, and flocculation (Ahmad *et al.*, 2014). Drying cell biomass after harvesting leads to higher yield during extraction compared with disrupted, cell biomass (Ageitos *et al*., 2011). numerous methods have been invented, such as high-pressure homogenization, ultra-sonication, microwave treatment, to efficiently disrupt wet microbial cells so that high yield can also be obtained during extraction, the soxhlet method are traditionally used for extraction using organic solvents such as methanol, or hexane (de Boer *et al*., 2012). A portion of the microbes is recycled after fermentation, while the remaining microbes processed to produce amino acids or peptides, which have wide applications (e.g., biomaterials, bioplastic, and biofoam) and generate more revenue for the biorefinery. Oligosaccharide-consuming heterotrophic microbes are reducing the production cost, technoeconomics Economics is the key to a successful pross (Yang and Wyman, 2008).

Materials and Methods

Culture Conditions

An optimally high carbon-to-nitrogen (C: N) ratio is key to allowing cells to reach their maximal lipid storage capacity (mass lipid per dry cell mass) and yield (lipid produced per carbon source consumed). Lipid accumulation per liter of culture is usually optimal at molar C: N ratio exceeding 65 and near 100 (de Boer *et al*., 2012) Phosphorous and sulfur limitations have been recently applied to enhance lipid accumulation in temperature 20–28C for molds and optimal growth pH ranges is 4–7 for yeasts and molds (Wu *et al*., 2011). SCO production from cellulosic substrates Biochemical process for conversion of cellulosic biomass to lipid. The biochemical fermentation routes of sugars to lipids have been recently applied to enhance lipid accumulation because of potential difficulty in reducing the nitrogen content of certain biomass substrates (Wu *et al*., 2011). The cultures were selected from those samples used in the previous study and are designated by the same numbers, the floats on the surface of medium that it presents in surface of contact. A volume of 150 ml of media contained in a 200 ml. flask was used in all of the experiments here reported. The cultures were grown at 25°C .To determine the quantitative relations between the products formed, by drained off and the wrinkles growth fungus (fresh and dry weight the mycelium repeatedly washed. The same cultures are seen in grown upon a medium containing cellulosic garbage cartoon container, in the beginning it was hoped that *Aspergillus niger* cultures could be divided into two general groups, one of which produced citric acid and the other oxalic acid. This would lend some aid to the problem of classifying this puzzling group of black Aspergilli. In this respect the data are disappointing. No cultures produced citric acid only under all conditions or oxalic acid only under all conditions.

TLC analysis of Pretreatment Step

Analyses was to determine the real lignocellulosic hydrolysates which achieved step 2, 10 ml of hydrolysates solution was centrifuged at 2500 g for 10 min to achieve a good phase separation and then suspended in 1 ml transferring to TLC plate as spots. Later put TLC plate in glass jar containing 200 ml of hexane: diethyl ether: acetic acid (80:20:1, v: v) (Wu *et al*., 2010).

Potato Dextrose Agar (PDA) and Broth

To prepare potato infusion, boil 200 g sliced, unpeeled potatoes in 1 liter distilled water for 30 min. Filter through cheesecloth, saving effluent, which is potato infusion. Mix with Dextrose 20g, Agar and Water and boil to dissolve. Autoclave 15 min at 121°C. Dispense 20-25 ml portions into sterile 15 × 100 mm petri dishes. Final pH, 5.6 ± 0.2.In broth medium without agar. The modified medium replaced the carbon source (Dextrose) with cellulosic garbage such as cartoon container in concentrations (0.5, 10, 20)% (Algam, 2010).

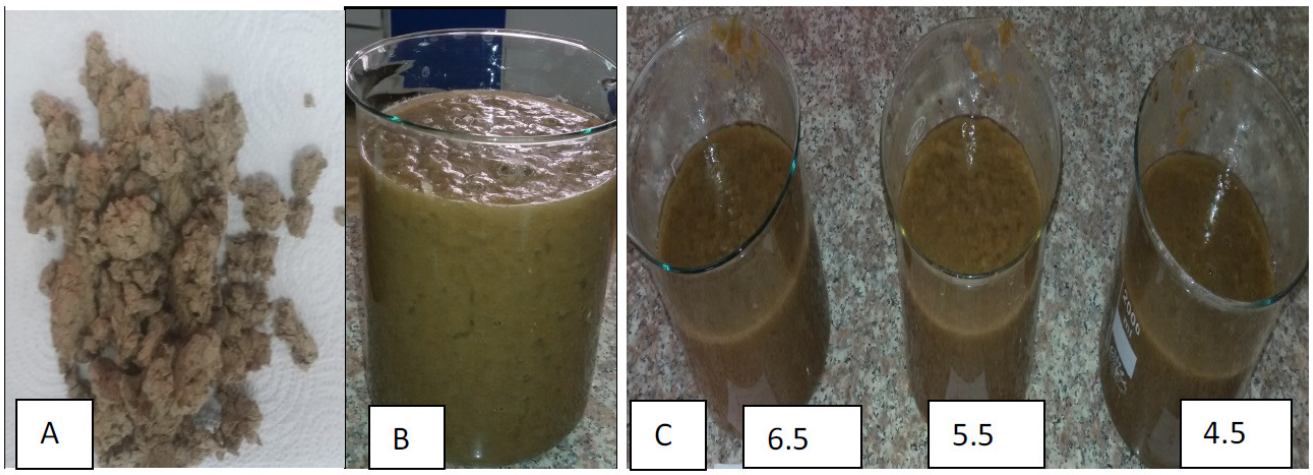


Fig. 3 : cellulosic garbage A-Grinding B- garbage with dilute acid after boiling C- PH value

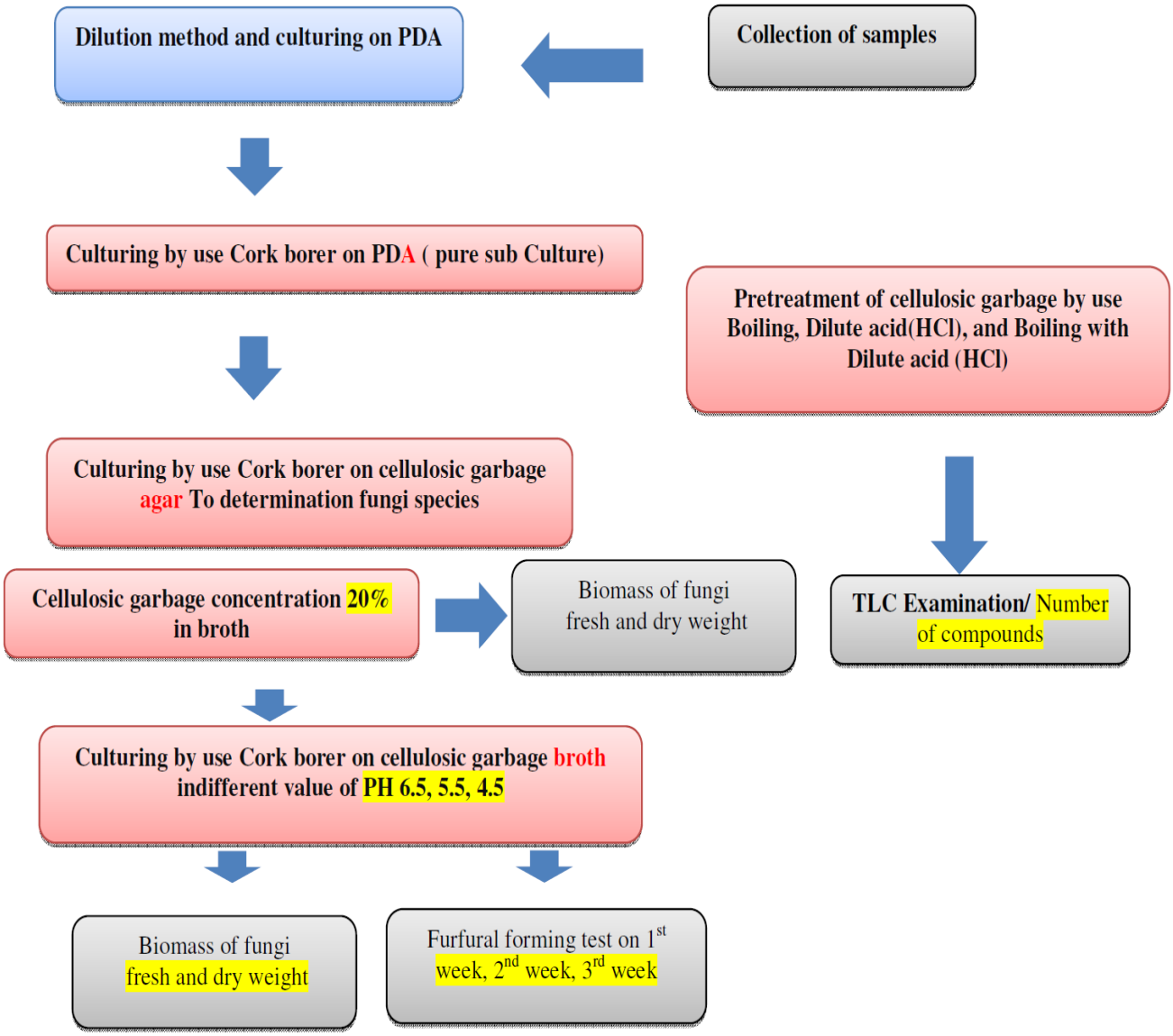


Fig. 4 : Experimental steps

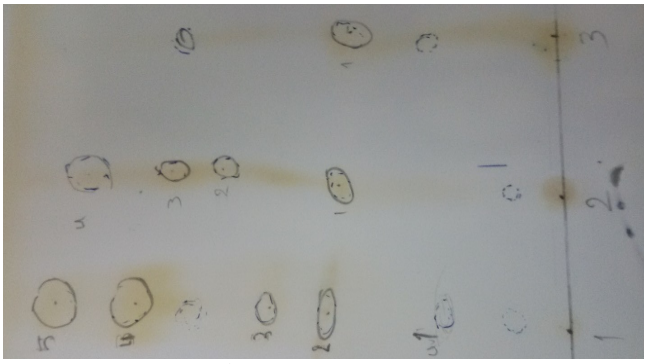
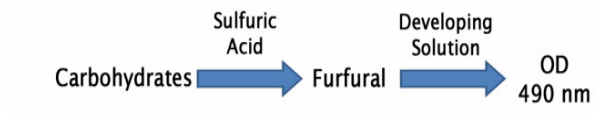


Fig. 5 : TLC SPOTS

Total Carbohydrate Assay Kit

Glucose Standards for Colorimetric Detection Add 0, 2, 4, 6, 8, and 10 of the 2 mg/mL standard solution directly into a 96 well plate, generating 0 (blank), 4, 8, 12, 16, and 20 g/well standards. Add water to each well to bring the volume to 30. Sample Preparation Tissue (50 mg) or cells can be homogenized in 200 of ice-cold Assay Buffer. Centrifuge the samples at 13,000 for 5 minutes to remove insoluble material. Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve. Bring samples to a final volume of 30 with water. Incubate the Assay reaction for 15 minutes at 90 Cover the plate and protect from light during the incubation. Then Add 30 of Developer to each well. Mix well using horizontal shaker for 5 minutes at room temperature. Mix contents for 1 minute before measuring the absorbance at 490 nm (A490).



Collection of samples

Serial	Samples Source	No.	Place
S1	Contamination fruits	**10**	Addle area
S2	Agricultural Soil	10	Addle area
S3	Submerged garbage	**10**	Addle area

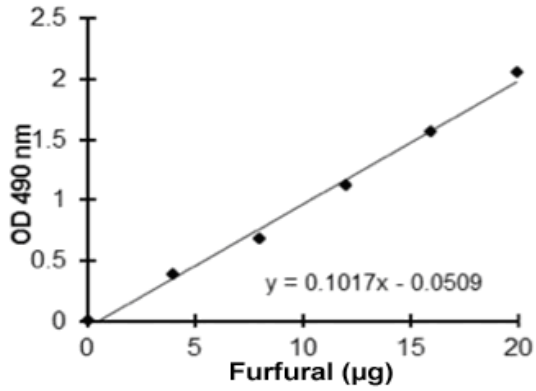


Fig. 6 : Show the Standard curve furfural and type of sample

Results and Discussion

Dilute acid pretreatment has been used for many years and has recently been intensively investigated. In this method, acid is used in a low concentration at a high temperature to dissolve hemicelluloses from biomass cell walls, rendering celluloses more accessible to enzymes this result with (Roche *et al.,* 2014). Also Pretreatment using dilute acid is favorable for scale-up production of biofuels because of its high efficiency to convert most of the hemicellulose into soluble sugars and the use of cheap chemicals (Balan, 2014).

(Pretreatment type	**Dilute acid**	**Boiling**	**Dilute acid + Boiling**
N0. Of spots in TLC	**5**	**3**	**7**

Fig. 7 : Show the No. of spots on TLC of Pretreatment type

Various forms of stress of pH value (figure 2) can reduce bulk translation activity and consequently elevate levels of no translating mRNAs, , and acidic stress can induce P-body formation without pronounced repression of translation and SG formation, the physiological effects of furfural and HMF on yeast cells, and also suggest the potential usefulness of cytoplasmic mRNP granules as a warning sign or index of the deterioration of cellular physiological status in the fermentation of lignocellulosic hydrolysates (Kim, *et al*., 2013). Also (Fig. 3) the incubation time significant influence on the mean on the furfural production as we are seeing, the second week the highest furfural production compare with other weeks.

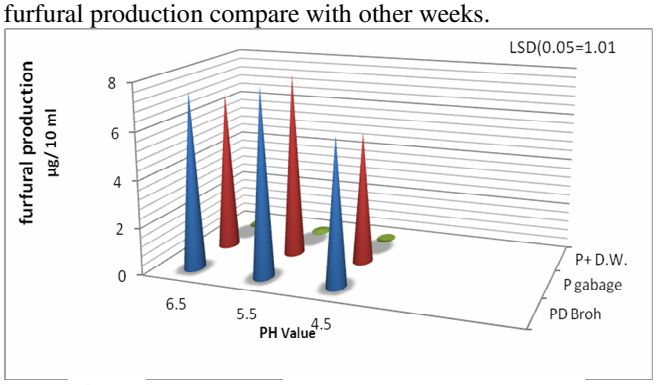


Fig. 8 : Effect of pH value on furfural production

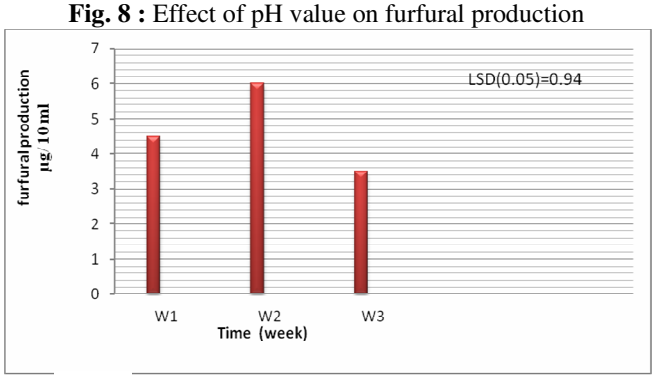


Fig. 9 : Dhow the incubation time influence furfural production

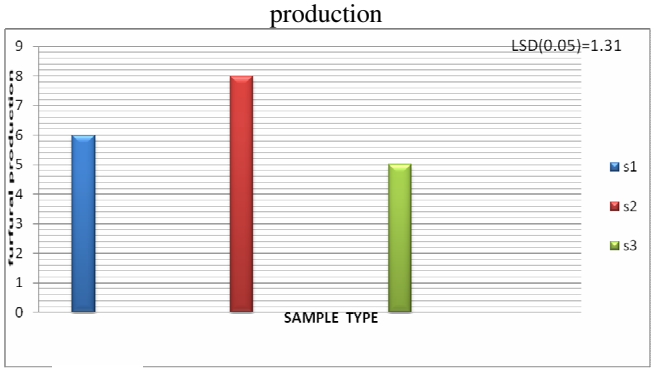


Fig. 10 : Show the sample type influence on furfural production µg/ 10 ml

The production of new secondary metabolites from previously silent gene clusters. Cultivation of other species of the black aspergilli showed that raisins induced sclerotium formation by *Aspergillus niger*. Even though many secondary metabolites have been reported from *Aspergillus niger*, this species has never been reported to produce aflavinin related indoloterpenes .When the fermentation broth of *A. fumigatus* was screened, a number of indolic alkaloids with antimitotic. The improvement of the accessibility of feedstocks by removing hemicelluloses and increasing surface areas to allow enzyme penetration, steam explosion pretreatment has advantages and limitations. Steam rapidly heats the biomass to the target temperature without excessive dilution of the resulting sugars this method uses less hazardous chemicals and conditions, reducing the environmental impact, however, the steam explosion method does have its drawbacks, such as incomplete disruption of lignin and the generation of toxic chemicals, which may affect the downstream processes (Buchan, 2008).

Conclusions

The major points that can be concluded from the present study are: Using the cellulosic garbage as carbon source by fermentation and Stimulate the microorganism to furfural production. Furfural production increased at treatments 5.5 of pH. after 14 days of incubation.

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