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Optimizing Growth Conditions Provoked Ethanol Production by Fungi Grown on Fructose

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ABSTRACT

Nine fungal strains known for their cellulolytic activities including: *Aspergillus oryzae*, *Aspergillus versicolor*-I, *Aspergillus versicolor*-II, *Fusarium oxysporum*, *Fusarium oxysporum*-I, *Mucor indicus*, *Penicillium citrinum*, *Phanerochaete chrysosporium* and *Pleurotus sp*., were tested for their fermentation capabilities on Mandels medium supplied with 10% fructose as sole carbon source at 35°C under minor air conditions. Among the nine strains *F. oxysporum* recorded the best ethanol production to be 0.077g ethanol / g carbon. Mandels medium components were further modified individually for best ethanol production. Throughout nutritional development the ethanol yield efficiency increased from 15 up to 44%, using yeast extract as sole nitrogen source at C:N ratio of 2:1 and initial pH=5. Fructose was tested at concentrations of 5%, 10%, and 15%, altered ratio between fructose consumed in ethanol production and that in fungal growth to be 2.2:1, 1.1:1 and 1:1.8, respectively, of which 5% fructose was considered the best in spite of 10% fructose that gave fermentation yield efficiency of 44.9%.

Key words: Ethanol production, fructose, *Aspergillus oryzae*, *Aspergillus versicolor*, *Fusarium oxysporum*, *Mucor indicus*, *Penicillium citrinum*, *Phanerochaete chrysosporium*, *Pleurotus sp*.

Introduction

Through many decades, non resting studies have been held up till now to effectively substitute depleting fossil fuels with biofuel from renewable resources for environmental and economical aspects. The agricultural and agro-industrial wastes have been proclaimed for this purpose. Agricultural wastes depend on debris pro-harvesting crops that are rich in ligno-cellulosic materials. On the other hand, the agro-industrial wastes depend on peels, pulps, juices and washing water resulting from processing legumes, fruits and vegetables that are rich in sucrose, glucose and fructose sugars content beside polysaccharides like cellulose and starch making their high content of fermentable carbohydrates an added value. In average, fructose, glucose, sucrose and total sugars constitute 2-7%, 1-4%, 0.1-8% and 4-13% of fruits (W/W), respectively, while in vegetables they constitute 0.5-2%, 0.02-2%, 0-4% and 0.5-5.5%, respectively (Li *et al*., 2002). Statistics confirmed that sugar crops, fruits and vegetables occupy nearly 35% of total of 6,940,000 ha (16.5 million feddan) of cropping area in Egypt, of which considerable amounts are industrially processed (FAO, 2009).

Many researchers have successfully hydrolyzed orange and grapefruit peel wastes using commercial cellulases and pectinases to glucose, fructose and other sugars, as stated by Grohamann *et al*. (1994a, 1995) and Wilkins *et al*. (2007a). Grohamann *et al.* (1994b) reported that the recombinant *Escherichia coli* produced 0.41g ethanol/g sugars in orange peel hydrolysate, which in the same time contained galacturonic acid which could efficiently be fermented to ethanol by the bacterium too. Approximately 50-60% of citrus fruits are peel wastes, of which the hydrolyzed orange peels have been fermented successfully by *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* to produce 37 and 40 g/L ethanol of 80 ad 88% of theoretical yield, respectively (Wilkins *et al*., 2007b). It is worthy to notice that orange peel waste contains 17% soluble sugars, as reported by Rivas *et al*. (2008). Fish *et al*. (2009) worked on watermelon crop, as they stated that 20% were left in field being misshapen and of surface blemishes, getting worthy of its juice that contained 7-10% (W/V) of directly fermentable sugars (glucose, fructose and sucrose) and 15-35 mol/100ml free amino acids. They used the juice as diluent and supplement in fermentation of molasses by yeast to produce successfully ethanol at a yield of 41-46%. Alone, watermelon juice must be concentrated 3 fold to serve as the sole feedstock for ethanol biofuel production.

Thus in the present study, there is a great need to emphasize the role of some cellulytic fungal strains in fermenting fructose (ketohexose) in its free form under minimum aeration, as well as their role in fermenting glucose (aldohexose) had been studied before by Sarabana *et al.* (2015). This study was crucial for setting up

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futuristic consolidating bioprocessing studies that uses cellulytic fungi in breaking down agro-industrial wastes and fermenting resulting glucose and fructose contents to ethanol biofuel.

Materials and Methods

Fungal strains:

Nine fungal strains known for their cellulolytic activity (Sarabana *et al.,* 2014 and Abou El-Khair *et al.,* 2014) were tested for their ability to ferment fructose, as an abundant hexoketose sugar in agro-industrial wastes, to produce considerable amounts of ethanol. The nine fungal strains used were *Fusarium oxysporum*-I, *Mucor indicus*, *Aspergillus versicolor*-I, *Aspergillus versicolor*-II, *Aspergillus oryzae*, *Penicillium citrinum*, *Fusarium oxysporum*, *Phanerochaete chrysosporium* and *Pleurotus sp*. All fungal strains were generously offered by Microbiology Research Department (SWERI-ARC), Giza, Egypt.

Starter medium:

All strains were enriched in Mandels medium (Mandels *et al.,* 1974) on orbital incubator shaker at 35˚C (125 rpm) for 5 days. The medium contained the following ingredients (g/L): urea, 0.3; $(NH_4)_2$ SO_4, 1.4; glucose, 10; $MgSO_4.7H_2O$, 0.3; KH_2PO_4, 2; $CaCl_2.2H_2O$, 0.3; Bactopeptone, 1; Tween 80, 0.1; trace elements: $FeSO_4.7H_2O$, 5mg; $MnSO_4.H_2O$, 16mg; $ZnCl_2.2H_2O$, 17mg; $CoCl_2.6H_2O$, 2 mg. The medium, trace elements and glucose were autoclaved separately (121°C for 15 min).

Fermentation medium (FM):

The enriched cultures from the tested fungal strains were maintained to 0.5 % dry biomass (w/v) and were used for inoculating (5%, v/v corresponding to 0.1g dry weight/20ml) the FM volume of 150ml medium in 200 ml firmly closed bottles to maintain minimized aeration conditions for fermentation process (Kenealy and Dietrich, 2004). The FM structure was based on modified Mandels medium (Fatma *et al.,* 2010) with further modifications as follows: Fructose 100g/L as the sole C source to supply 4% Carbon (w/v), $(NH_4)_2SO_4$ 47 g/L as the sole N source to supply1% nitrogen (w/v), 0.1% yeast extract and initial pH was adjusted at 5. Inoculated fermentation bottles allocated in a complete randomized design with three replicates were statically incubated at 35˚C for 14 days and samples were collected at two days intervals for further studies.

Optimization of fermentation medium:

To improve ethanol production, FM contents were substituted individually. Nitrogen source content in control (designated as ammonium sulfate) was substituted with organic and inorganic nitrogen sources (ammonium phosphate, casein, peptone, potassium nitrate and yeast extract), fulfilling the same total N% (Pasha *et al.,* 2012). Afterwards, the best N source was validated in different C:N ratios test (2:1, 4:1, 6:1 and 8:1), followed by testing the best C:N ratio at different initial pH values (4, 5 and 6), adjusted by HCl/NaOH solutions (0.1N). Based on best former parameters, carbon level test was conducted at levels of 50, 100 and 150 g/L fructose and with fixed inocula dry weight and size volume.

Analysis:

Biomass was determined gravimetrically (DW) in the collected samples. The fungal mycelia were harvested every 48 h during fermentation, separated by filtration through a pre-dried and weighed filter paper (Whatman No.1), repeatedly washed with distilled water, dried at 70°C overnight and dry weight was calculated (Srivastava *et al.,* 2011).

Fructose utilization was periodically measured according to resorcinol method described by Sadasivam and Manickam (2004).

Ethanol spectrophotometric determination done according to dichromate method (Caputi *et al.,* 1968). Ethanol production was expressed as g ethanol/g carbon added in the FM.

The basics of the following calculations were clarified by Hatzis *et al*. (1996):

$$\text{Ethanol production yield : Actual Yield} = \frac{\text{Ethanol (g/L) x100}}{\text{Initial Fructose (g/L)}}$$

Ethanol yield efficiency: Yield Efficiency % = $\frac{\text{Actual Yield x100}}{\text{Theoretical yield}}$

Where the theoretical yield % of ethanol from hexoses = 51.2%

Fructose % consumed in ethanol production: $FOH\ \% = \frac{Ep \times 180}{92} \times \frac{100}{Ft}$

Where:
FOH is Fructose consumed %
Ft is the total consumed fructose (g/L)
Ep is the ethanol produced (g/L)
Fructose % consumed in growth: $Fg\% = 100 - FOH\%$

Statistical analysis:

All results data were accomplished in triplicates and statistically evaluated by least significant differences (LSD) in one way completely randomized analysis of variance (ANOVA) at significance 5% calculated using CoStat (6.311) software (Maruthai *et al*., 2012).

Results and Discussion

I- Ethanol production among fungal strains:

The fungal production of ethanol on FM, substituted with fructose as the sole carbon source, was considered as the preliminary test differentiating ability of those fungal strains in fermenting fructose to ethanol under static conditions (minimum air requirements) in accordance to fermentation time (days). As shown in Fig. (1), *F. oxysporum* produced 0.077 g ethanol/g carbon after 8 days of static incubation and considered the best ethanol producer, at LSD of 0.014. In the second rank, both *Pleurotus* sp. and *A. oryzae* produced 0.062 g ethanol/g carbon after 8 and 10 days, respectively. The production peaks by most of fungal strains were characterized by more than one maximum production point gapped by 2 days drop.

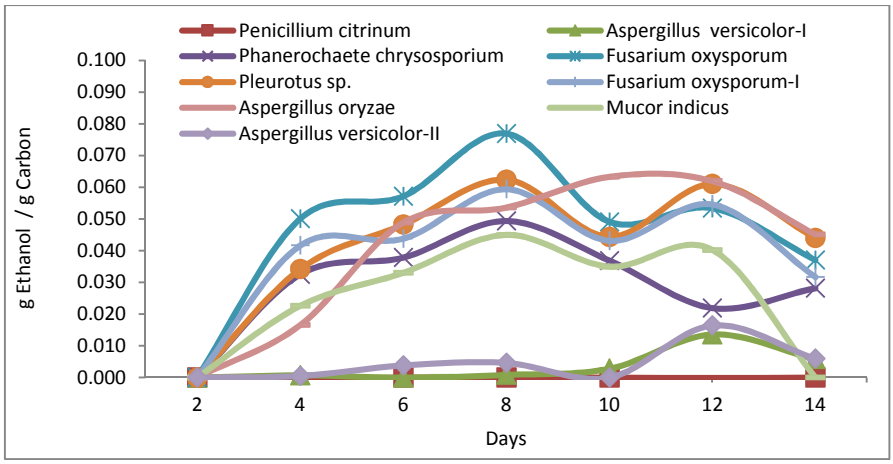

Fig. 1: Ethanol production by nine fungal strains grown on fructose as sole carbon source.

The capability of several fungal strains to utilize hexoses under minimum aeration had been demonstrated in many researches. Gunner and Alexander (1964) stated that *F. oxysporum* in spite of being strictly aerobic, it can proliferate anaerobically. Cochrane and Cochrane (1966) found that *Fusarium solani* anaerobically ferment glucose and that specific activity of enzymes responsible for accelerating metabolic systems increased 2-8 folds, especially those involved in glycolysis. Besides, Anasontzis *et al.* (2014) found that *F. oxysporum* characterized by over expression of phospho-glucomutase in glycolysis pathway and consequently enhanced its growth depending on available hexoses which was not found in *Saccharomyces cerevisiae.* On the other hand, Suihko (1983) studied the ability of *F. oxysporum* to utilize several sugar types anaerobically and found that it fermented glucose, mannose and galactose as aldohexoses and fructose as ketohexose to ethanol more rapidly than other sugars including pentoses, sugar alcohols, sugar acids, oligosaccharides, polysaccharides and organic

acids. Gangadhara *et al.* (2010) stated that five isolates of *F. oxysporum* gave best growth with fructose than glucose.

Pushalkar and Rao (1998) clarified the impact of utilizing different hexoses, pentoses and disaccharides as substrates on *Aspergillus terreus* growth and ethanol production during 7 days in non aerated fermentation, in which glucose followed by fructose were found to be the best in ethanol production. Kenealy and Dietrich (2004) found that *Phanerochaete chrysosporium* survived transient oxygen limitation by fermenting glucose but didn't grow and this may emphasize the decline in ethanol production after 8 days of utilizing fructose under minor air condition in present study that might be due to lag phase. In a comparative study, Abedinifar *et al.* (2009) found that *Mucor indicus* was better than *Rhizopus oryzae* but less than *S. cerevisiae* in fermenting rice straw hydrolysate full of aldohexoses anaerobically to ethanol at a yield of 0.43 g ethanol/ g hexose. Under minimized aeration, *Mucor indicus, A. versicolor*-I, *A. versicolor*-II and *P. citrinum* proved that they were not worthy competitive in ethanol production utilizing fructose in the present study or even with glucose as previously stated by Sarabana *et al.* (2015).

II- Optimization of FM for maximizing ethanol production:

Effect of nitrogen source:

F. oxysporum, *Pleurotus sp.* and *A. oryzae* were the most reliable fungal strains to test their maximum ethanol production possible in accordance to nitrogen source type. Nitrogen source in control (designated as ammonium sulfate) was substituted with ammonium phosphate, casein, peptone, potassium nitrate and yeast extract, fulfilling the same total N% (Pasha *et al.,* 2012). Among the best three fungal ethanol producers, *F. oxysporum* proved to be the ultimate ethanol producer statistically at LSD of 0.064. *F. oxysporum* ethanol production recorded 0.475 and 0.305 g ethanol/g carbon after 4 and 8 days, respectively, using yeast extract as sole nitrogen source, while with ammonium phosphate it recorded 0.225 g ethanol/g carbon after 4 days (Fig. 2).

Either nitrogen sources offered *F. oxysporum* maximum production 4 days earlier than the control (ammonium sulfate). It was worthy to notice that the increments previously noted in ethanol production had been followed by dramatic drops for 2 days. On the other hand, *Pleurotus sp.* and *A. oryzae* gave their best shots 76% and 84% less than maximum ethanol produced by *F. oxysporum* when utilized casein and ammonium phosphate as sole nitrogen sources, respectively.

Gunner and Alexander (1964) studied the effect of salts (as: sulfur), ions (as: nitrate, ceric or ferric), MnO_2 and yeast extract (containing unidentified active substances) individually in *F. oxysporum* growth medium. These substances have an oxidation reduction potential that could act as terminal electron acceptors in absence of oxygen and could penetrate inside the fungus cells to exist at a higher potential than those coenzymes that must be oxidized. The fungus was grown anaerobically on glucose and proved to proliferate efficiently. Even under aerobic conditions, Sharma and Pandey (2010) ensured that *F. oxysporum* showed highest growth on yeast extract agar medium compared to other media. Added to that, Sorensen and Sondergaard (2014) stated that 4 different *Fusarium* strains recorded type and quantity differences in production of several known secondary metabolites influenced by difference in yeast extract brands used in growth medium. Apparently in present study and due to these explanations; yeast extract influenced and pushed the growth of *F. oxysporum* under minimum aeration in the first 8 days indicated by the increase in ethanol production more efficiently than other nitrogen sources.

Wu *et al.* (2003) stated that *Pleurotus sp.* mycelial growth was better with fructose than glucose. On the other hand, in case of ethanol production by fermentation, the bioconversion efficiency of fructose was better than of glucose especially when yeast extract was used instead of peptone as sole nitrogen source. This can spot light on the effect of altering nitrogen source on utilizing different hexoses can affect the growth of *Pleurotus sp.* and consequently the resulting metabolites. Neelman *et al.* (2013) indicated that *Pleurotus sp.* best mycelia yield was determined with casein than with peptone, yeast extract and ammonium salt. This emphasizes superiority of casein in FM over other nitrogen sources in maximizing ethanol production by *Pleurotus sp.* in present study. On the contrary, *Aspergillus oryzae* maximum ethanol production was humble recording below 0.08 g ethanol/ g carbon.

Fungi almost alter the pH of its growth medium. If nitrogen is provided as an ammonium salt the utilization of ammonium ion will make the medium more acidic. Alternatively, if nitrogen is provided as sodium nitrate the medium becomes more alkaline as the nitrate ion is removed (Landecker, 1982). This deduced results in the present work, as the three fungal strains were more productive to ethanol when nitrogen source used was either ammonium phosphate or ammonium sulfate than potassium nitrate due to predicted favorable alteration in pH of FM during their consumption.

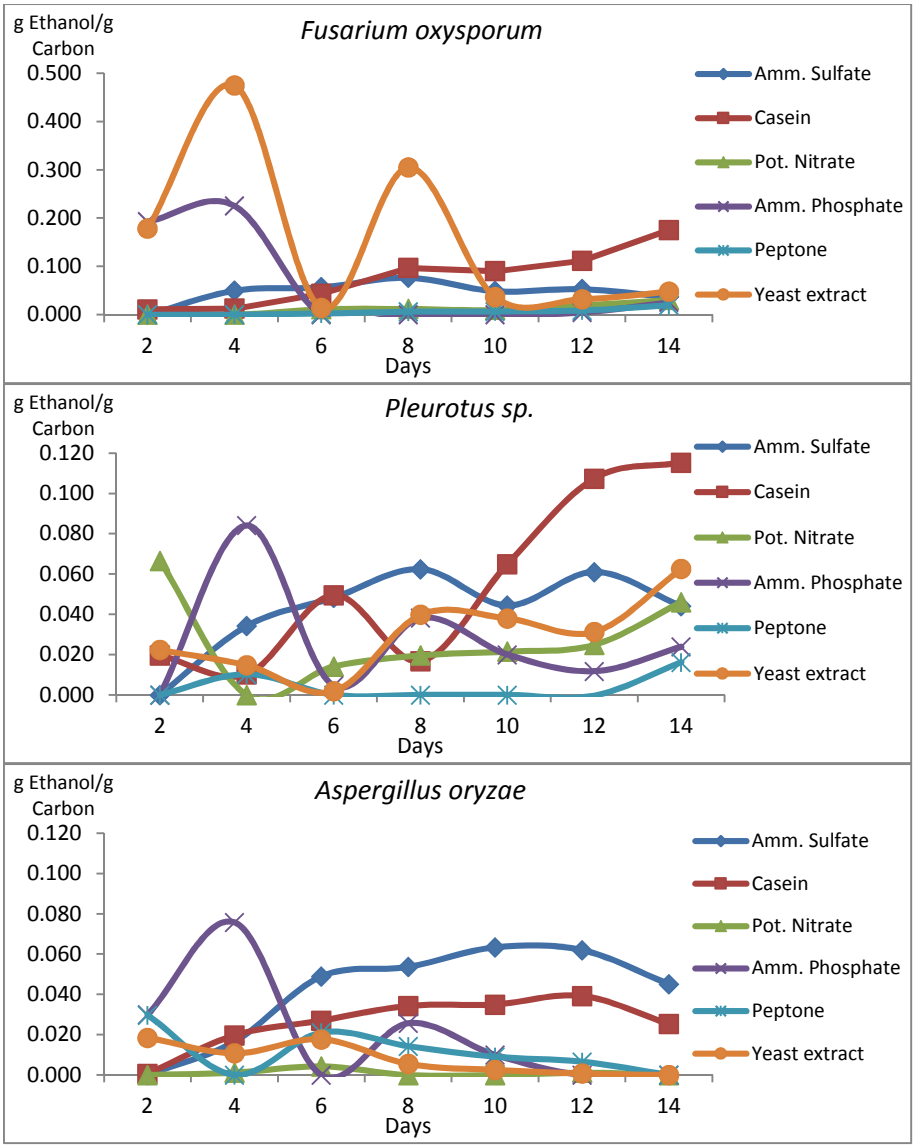

Fig. 2: Effect of different nitrogen sources on ethanol production by *F. oxysporum*, *Pleurotus sp.* and *Aspergillus oryzae*.

Effect of C/N ratio:

F. oxysporum utilizing fructose optimumly with yeast extract being the best sole nitrogen source in FM, different C:N ratios; 2:1, 4:1, 6:1 and 8:1 were tested (Fig. 3). *F. oxysporum* was found to produce more ethanol with narrow C/N ratio. Statistically at LSD=0.1471, C/N ratio of 2:1 was the best, as the fungi produced 0.558 g ethanol/g carbon at 8 days at one max peak. At this ratio the fungi maintained continuous production above 0.477 g ethanol/g carbon for 6 days, while followed by the ratio of 4:1where two max peaks (as before) at 4 and 8 days.

Taylor *et al.* (1995) studied continuous fermentation by yeast for ethanol production and found that it was depending on C:N ratio of 33:1. On the same trend, Manikandan and Viruthagiri (2010) stated that *S. cerevisiae* best ethanol production was maintained at C:N ratio of 35:1 in batch fermentation.

On the contrary, the filamentous fungi *Ph. chrysosporium* utilized glucose under oxygen limitation and increased its ethanol production when smaller C:N ratio was used. The fungal cells were characterized by detectable alcohol dehydrogenase in its extract which have crucial role in the formation of ethanol from acetaldehyde intermediate during fermentation (Kenealy and Dietrich, 2004). Also, Ruiz *et al.* (2007) found that *F. oxysporum* under limited aeration could ferment glucose at low C:N ratio of 6:1 efficiently. Besides, Asachi *et al.* (2011) used C:N ratio of 13:1 in sugar fermentation for ethanol production by *Mucor indicus*.

In the present study it is worthy to notice that less C:N ratio maintained more stable ethanol production by *F. oxysporum* because the abundance of nitrogen source in the form of yeast extract had crucial role in its cell metabolism and propagation, as well as importance of carbon in ethanol production by fermentation. Added to

that, Gunner and Alexander (1964) notified to the existence of unidentified active substances in yeast extract that acted as terminal electron acceptors in absence of oxygen and could penetrate inside the fungus cells to exist at a higher potential than those coenzymes that must be oxidized. So by increasing yeast extract share through lowering C:N ratio, led to abundance of those substances that ensured anaerobic growth on fructose and efficient proliferation. Consequently, fungal biomass development increased cumulative and continuous production of ethanol.

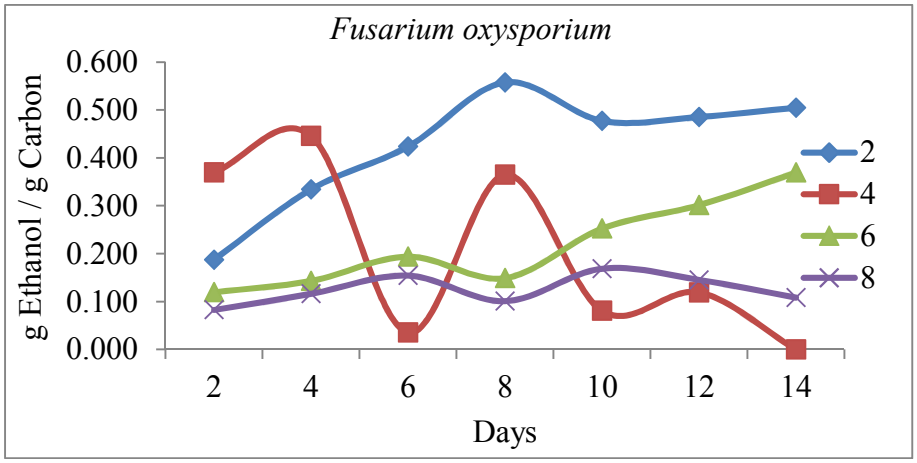

Fig. 3: Effect of C:N ratio on *F. oxysporum* ethanol production.

Effect of initial pH:

As the optimization process proceeded, the best production of ethanol by *F. oxysporum* using yeast extract as a sole nitrogen source at C:N ratio of 2:1, and was subjected to further optimization by altering the initial pH of FM. Different initial pH values of 4, 5 and 6 were tested (Fig. 4). The maximum production of ethanol was trapped between pH values 5 and 6 statistically (at LSD=0.0241) after 8 days being 0.631 and 0.596g ethanol/g carbon, respectively. The fungus went through the same production behavior at pH 5 parallel to that at pH 6, as both pH values ensured high continuous ethanol production after their maximum production recorded after 8 days. On the other hand no ethanol production could be detected at pH4.

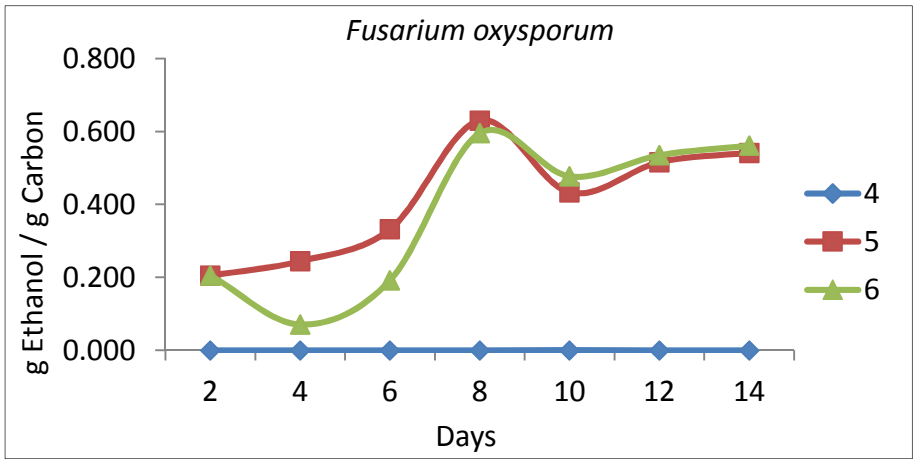

Fig. 4: Effect of initial pH value on *F. oxysporum* ethanol production.

Landecker (1982) stated that pH altered cell permeability, as at lower pH the protoplasmic membrane became saturated with hydrogen ions so that the passage of essential cations was limited. Even the external hydrogen ion concentration affected pH within the cell, which in turn affected enzyme activity, as enzymes were inactivated at either pH extremes. The unfavorable pH might alter normal synthetic ability of the cell.

Oritsejafor (1986a) found through studying the effect of pH on growth and survival of three isolates of *F. oxysporum* that the most favorable pH values for growth were between 5 to 7 and that chlamydospore germlings lyses was most pronounced at more acidic pH.

Gangahara *et al.* (2010) as well as Khilare and Ahmed (2012) stated that *F. oxysporum* optimum mycelial growth was at pH=6. Besides, Hossain *et al.* (2012) found that *F. oxysporum* optimum ethanol production was achieved anaerobically from fermenting glucose in media whose initial pH=6, while Sarabana *et al.* (2015) found it to be optimum at pH=5. On the other hand, *S. cerevisiae* best ethanol production was achieved at pH=5.5 (Manikandi and Viruthagiri, 2010).

According to the previously noted studies and present work results, *F. oxysporum* under partial limitation of oxygen could ferment hexoses optimally within pH values ranging from 5 up to 6, which it was mostly optimum for its growth too.

Effect of fructose initial concentration:

F. oxysporum proved to efficiently ferment fructose in FM containing yeast extract as a sole nitrogen source with C:N ratio of 2:1 at pH=5. The fungus ethanol productivity, mycelial weight propagation and pH in FM interactions as affected by fructose initial concentration of 50, 100 and 150 g fructose/L were studied. As shown in Fig. 5, *F. oxysporum* maintained same production behavior as before when its concentration was 10% (B), as it gave the best production of 0.574 g ethanol/ g carbon statistically at LSD=0.142. On the other hand, lowering fructose level to 5% (A) decreased ethanol production by 17% to be 0.475 g ethanol/g carbon with an obvious drop in activity between 6 and 10 days parallel to the drop in pH level.

Noticeably with both fructose concentrations A and B, the ethanol headed to maximum production with slight increase in pH and rise in mycelia weight (log phase). With further propagation in mycelial wt and after 6 days pH value in A and B decreased by 0.3 and 0.1, parallel to drop in ethanol production in A by 66% at 8 days before it rise again, while ethanol continued rising in B to maximum after 8 days. At the highest fructose concentration (C), an increase in mycelia weight with no valuable ethanol production took place. *F. oxysporum* succeeded to produce ethanol yield of 19, 23 and 4.8% with fructose initial concentrations of 5% (A), 10% (B) and 15% (C) g/L, after 6, 8 and 14 days, respectively, with concentration (B) scoring the highest yield as shown in Table (1). In a comparative study, Pushalkar and Rao (1998) stated that *A. terreus* under anaerobic conditions fermented fructose present in medium at concentration of 5% and gave ethanol yield of 2.2% after 6 days of fermentation.

From data in Table (1), total consumed fructose (%) included what was consumed in both growth and ethanol production recording 85% (average) of initial fructose used in FM for each concentration of A, B and C. By putting aside fructose % consumed in ethanol production we can predict that part % sharing in growth of fungal mycelia, putting in consideration the evolving CO_2. Depending on calculations, the approximate sugar sharing in growth was 31%, 48% and 89% of totally consumed fructose % (corresponding to 8, 41 and 114 g/L), while mycelia dry weight recorded 20, 35 and 60 g/L, in FM A, B and C, respectively, confirming that the more initial fructose concentration the more it was consumed in mycelial growth.

Briefly, results demonstrated that with 5% fructose the fungus utilized from it in fermentation twice that in growth, which is considered desirable if compared to 10% where the fungus used nearly equal amounts of fructose in fermentation and growth. Nevertheless, 15% fructose pushed the fungus metabolism to consume from it for growth 8 times that consumed in fermentation and ethanol production.

By comparing fermentation results due to increment in sugar concentrations used in A to B, the resulting accumulation of ethanol and acidity were unfavorable due to their toxic effect on fungal cells efficiency in fermenting sugar to ethanol, deviating sugar consumption to growth recording 31% up to 48% more than to ethanol production fermentation recording 69% down to 52%, respectively.

In case of (A) concentration, mycelial propagation interrupted with rise in ethanol production and pH. As mycelia growth regain its propagation again drop in both pH and produced ethanol were significant. On the contrary, with (B) concentration there was continuity in mycelial propagation with non significant drop in ethanol production. In C concentration, the mycelial propagation was nearly double of that in concentrations A or B.

Oritsejafor (1986b) found that increasing carbon concentration (sucrose) in growth medium increased *F. oxysporum* total growth, as carbon concentration was more important factor than C:N ratio of the medium in inducing chlamydospore production and reducing macroconidial formation of the fungus.

Guillaume *et al.* (2007) found in *S. cerevisiae* some types of hexose transport proteins named HXT had high affinity to glucose while others like HXT_3 had low affinity to glucose but worked on fructose transportation too. On other hand, Ali *et al.* (2013) declared that *F. oxysporum* had HXT with high affinity to glucose. Ethanol of concentration more than 3% acted as non competitive inhibitor for HXT causing its activity to decrease but *F. oxysporum* cells compensate this reduced activity by increasing HXT transcription and consequently its numbers.

Zhou *et al*., (2002) found that under anoxic conditions *F. oxysporum* consumed ethanol as an electron donor by catabolic oxidation to form acetate. Also, Huang *et al.,* (2015) stated that several organic acids suppressed growth of *F. oxysporum* specifically under anaerobic conditions where ethanol and organic acids

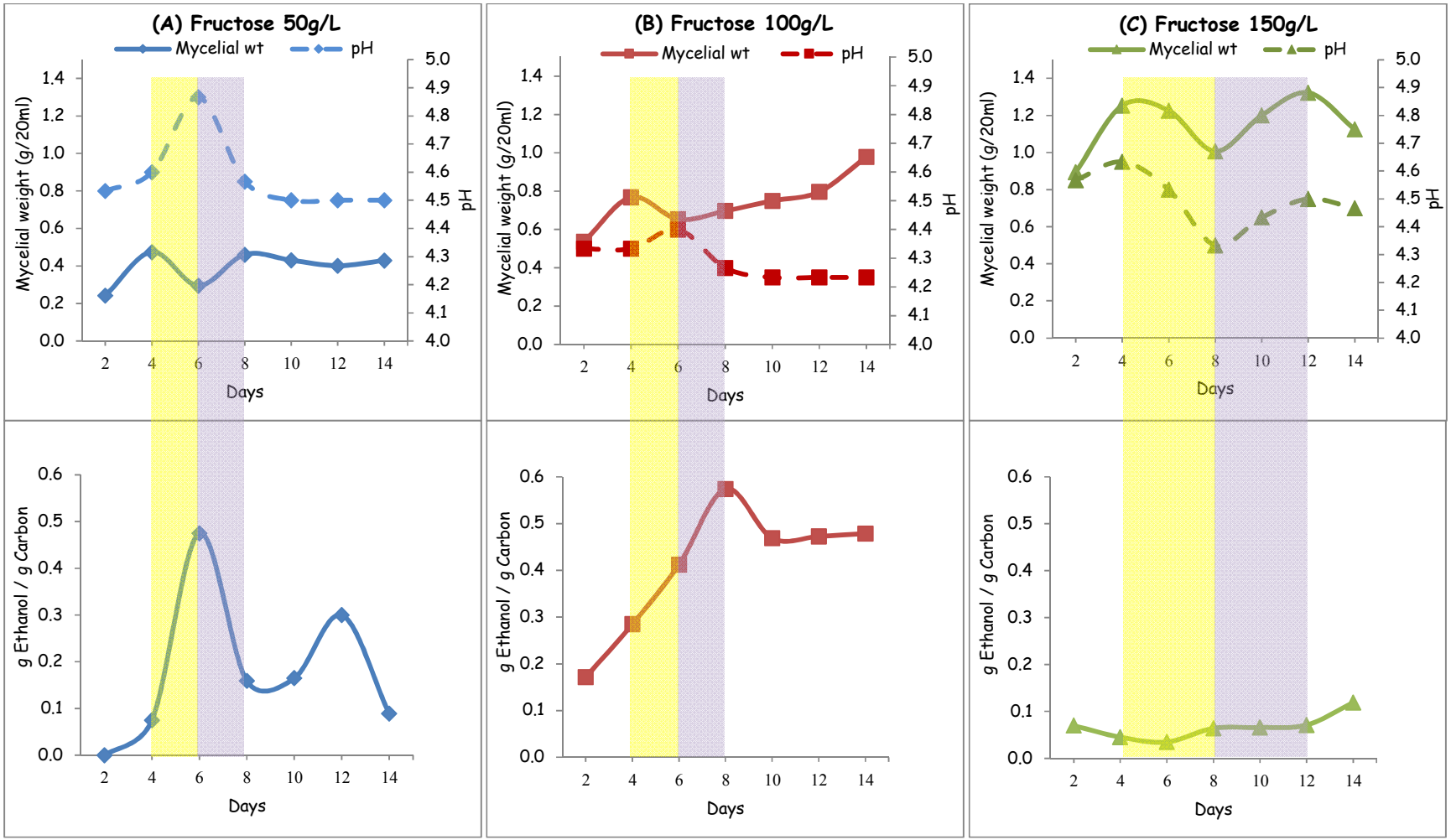

Fig. 5: Effect of fructose initial concentrations (A) 50 g/L, (B) 100 g/L and (C) 150 g/L on *F. oxysporium* propagation (mycelial wt), pH value and ethanol production during 14 days of fermentation.

were detected. Paschos *et al.,* (2015) confirmed the cumulative toxic effect of ethanol and organic acids on *F. oxysporum* during production of ethanol anaerobically. On the same trend, Gomaa (2012) stated that *Ph. chrysosporium* activity, growth, mycelia morphology and cell permeability was negatively affected by ethanol accumulation greater than 10g/L. That was why in the present work with (A) and (B) concentrations a recognizable drop in mycelial weight happened when ethanol increased between day 4 and 6 that altered pH value afterwards to be more acidic and lowering ethanol production. In spite of this, Anasontzis and Christakopoulos (2014) found that *F. oxysporum* was relatively tolerant to sugars and ethanol than other fungi but of lower conversion rate than yeast.

Table 1: Effect of initial fructose level on ethanol production

Initial fructose g/L		Fructose concentration after 14 days			Maximum ethanol production				Fructose Consumption		
		Residual g/L	Consumed g/L	Consumption %	g ethanol /g carbon	g ethanol /L	Actual yield %	Yield Efficiency %	in ethanol production F_{OH}%	in fungal growth F_g%	Ratio F_{OH}:F_G
A	50	23	27	85.2	0.475 (day 6)	9.5	19	37.1	69	31	2.2:1
B	100	14	86	86	0.574 (day 8)	22.96	23	44.9	52	48	1.1:1
C	150	22	128	85.3	0.119 (day 14)	7.14	4.8	9.4	11	89	1:8.1

Nevertheless, the rise in pH between days 4 and 6 in the three cases of fructose concentrations might be related to release of ammonium ion. Landecker (1982) stated that the release of ammonium ions from the deamination of amino acids and protein by fungi may cause the pH to rise.

That was why Taylor *et al.* (1995) recycled the contents of continuous fermentor held by yeast for ethanol production through stripping column in successful continuous operation for 150 days. The system allowed 100% and 90% conversion of 200 and 600 g/L glucose to eliminate the negative impact of ethanol and probable acidity accumulation.

Conclusion:

The efficiency of *F. oxysporum* in fermenting fructose under minimum aeration was optimally regulated by nitrogen source type (yeast extract), pH level (5), C:N ratio (2:1) and finally the fructose concentration used in FM that was critical as it led to best ethanol production and lowest fructose consumption in growth which was found to be in concentration of (A) 5% fructose, in spite of increase in ethanol yield in (B) than in (A) concentrations.

The success in choosing *F. oxysporum* among many fungal strains (known for their cellulytic activity) for this purpose was a fulfilled goal. As the tendency to use cellulytic action of this fungus in degrading cellulose content in seasonal agro-industrial wastes that are rich in fermentable sugar specially fructose before altering conditions to fermentation of fructose and other sugars freed by saccharification under anaerobic conditions for ethanol production more than to be utilized in growth (undesirable loss) under what so named consolidating bioprocessing.

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