# DEVELOPMENT OF BIOREACTOR TO ENRICH THE PROTEIN OF AGRICULTURAL RESIDUES

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## ABSTRACT

*The present study aims to develop a simple bioreactor to enrich the protein of rice straw using solid-state fermentation. laboratory experiments were carried out at Testing and Research Station for Tractor and Farm Machinery, Alexandria governorate, Egypt to study the effect of three bioreactor temperature levels $T_1$ (25°C), $T_2$ (35°C), and $T_3$ (45°C) and three rice straw moisture levels $M_1$(25%), $M_2$(50%) and $M_3$(65%) on fungus growth activity on rice straw under solid state fermentation by* ***Trichoderma harzinaum F-418 fungi (TH)*** *and molasses, valvic solution additive with controlled temperature and moisture at fermentation time (1, 2, 3, 4, and 5 days) to determine the optimum conditions of fungal treatment in terms of moisture and temperature. Parameters related to microbial growth crude protein content (CP%), and crude fiber (CF%) were measured every 24 hours and compared with crude protein content % (CP%), and crude fiber % (CF%) of rice straw before treatment (control treatment). The obtained results can be summarized as follows: The highest crude protein and the lowest crude fiber values were obtained at the temperature of 35 °C and the moisture content of 65% after 4 and 5 days. The best value of crude protein was 5.88% which increased 103% of the control treatment and the best value of crude fiber content was 28.35% by decreasing 30% of the control treatment. It is evident that these values demonstrated good operation of fermentation in bioreactor.*

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## 1. INTRODUCTION

There is a wide gap between animal feeds requirements and the available. The available local feeds are not sufficient completely to cover the nutritional requirements of the Egyptian livestock. Crop residues from rice plantation such as rice straw, rice husks are abundant in Egypt. Rice straw is unique relative to other cereal straws being high in silica and lignin with low digestibility and protein content and contains considerable amounts of cellulose and hemicellulose about 65% of the total dry weight and 18% lignin**.** Rice straw in developing countries is used as a main feed for ruminants **(Safa et al. 2011)**.

**Hanafi et al. (2012)** concluded that although several treatments have been used to improve the degradability and voluntary intake of rice straw, such as physical or chemical treatments, the practical use of these treatments is still restricted in terms of safety concerns, costs and potentially negative environmental consequences. **Hathout and El-Nouby** (**1977**) pointed that the aim of mechanical treatments is to increase the digestibility of roughages and to increase the quantity which can be eaten by animals without any harmful effect on their weight gain or productive performance. Mechanical treatment like grinding can improve the digestion of roughages because this treatment increase the surface area of roughages for rumen microbes digestive enzymes and increase voluntary intake. **Jonathan *et al*. (2012)** investigated the bio-conversion of sorghum and rice straw into value-added ruminant feed using ***Pleurotus pulmonarius*** in solid state fermentation over a period of forty days. The results obtained show a high positive correlation in the degradation of sorghum stalk and rice straw with an increase in the fermentation period. Sorghum stalk showed high digestibility compared to rice straw used for this study. The chemical composition results showed significant differences of ($P<0.05$), and high digestibility for the two substrates as the days of fermentation increases. **Roussos et al. (1993)** developed a pilot-scale packed-bed bioreactor with internal heat transfer plates, called the "Zymotis" bioreactor. The outer casing was acrylic, and it was 65 cm high, 50 cm wide, and 40 cm deep from front to back. This gave a total volume of 130 L, with a working capacity of 100 L. The aeration rate was varied from 0.1 to 0.2 L $h^{-1}$ / g-dry-substrate.

**Robinson and Nigam (2003)** mentioned that there are three basic groups of reactor exist for solid state fermentation (SSF), these May be distinguished by type of mixing and aeration used. In laboratory scale, SSF occurs mainly in flasks and the following reactors used for larger scal product formation. They also added that Packed bed reactors, usually in the form of a column, have emerged over the past 20 years as a potential alternative to the previously mentioned reactors. Disadvantages associated with packed bed column bioreactors for SSF include difficulties in obtaining the product, non-uniform growth, poor heat removal and scale-up problems. **pandey et al. (2001)** mentioned that the use of tray fermenters in large scale production is limited as they require a large operational area and tend to be labor intensive with mechanical handling also being difficult. It can be seen that the lack of adaptability of this type of fermenter makes it an unattractive design for any large-scale production. They added that growth of the inoculums in drum bioreactors is considered to be better and more uniform than that in tray fermenters. Fungal cultures are prone to damage in both rotating drum bioreactors and mixed reactors, as the increased sheer forces through mixing affecting the ultimate product yield being compromised. Although the mass heat transfer, aeration and mixing of the substrate is increased, in drum reactor, damage to inoculums and heat build-up through sheer forces may affect the final product yield. Application of drum reactor for large-scale fermentation also poses handling difficulties. **Stuart et al. (1999)** showed that drum bioreactors are designed to allow adequate aeration and mixing of the solid, whilst limiting the damage to the inoculums or product (sheer forces or heat build-up). Mixing and aeration of the medium has been explored in two ways by rotating the entire vessel or as showed by **Negal et al. (2001)** by various agitation devices such as paddles and paffles. **Lonsane et al. (1992)** concluded that rotating or the use of agitation can be carried out on a continuous or periodic basic, promoting surface mass heat transfer and a more uniform distribution of nutrients.

The objectives of the present study were:

1. To develop a new bioreactor for protein enrichment of agricultural residues.

2. To determine the effect of biological treatments on the chemical composition of treated rice straw in terms of temperature, moisture content and fermentation time.

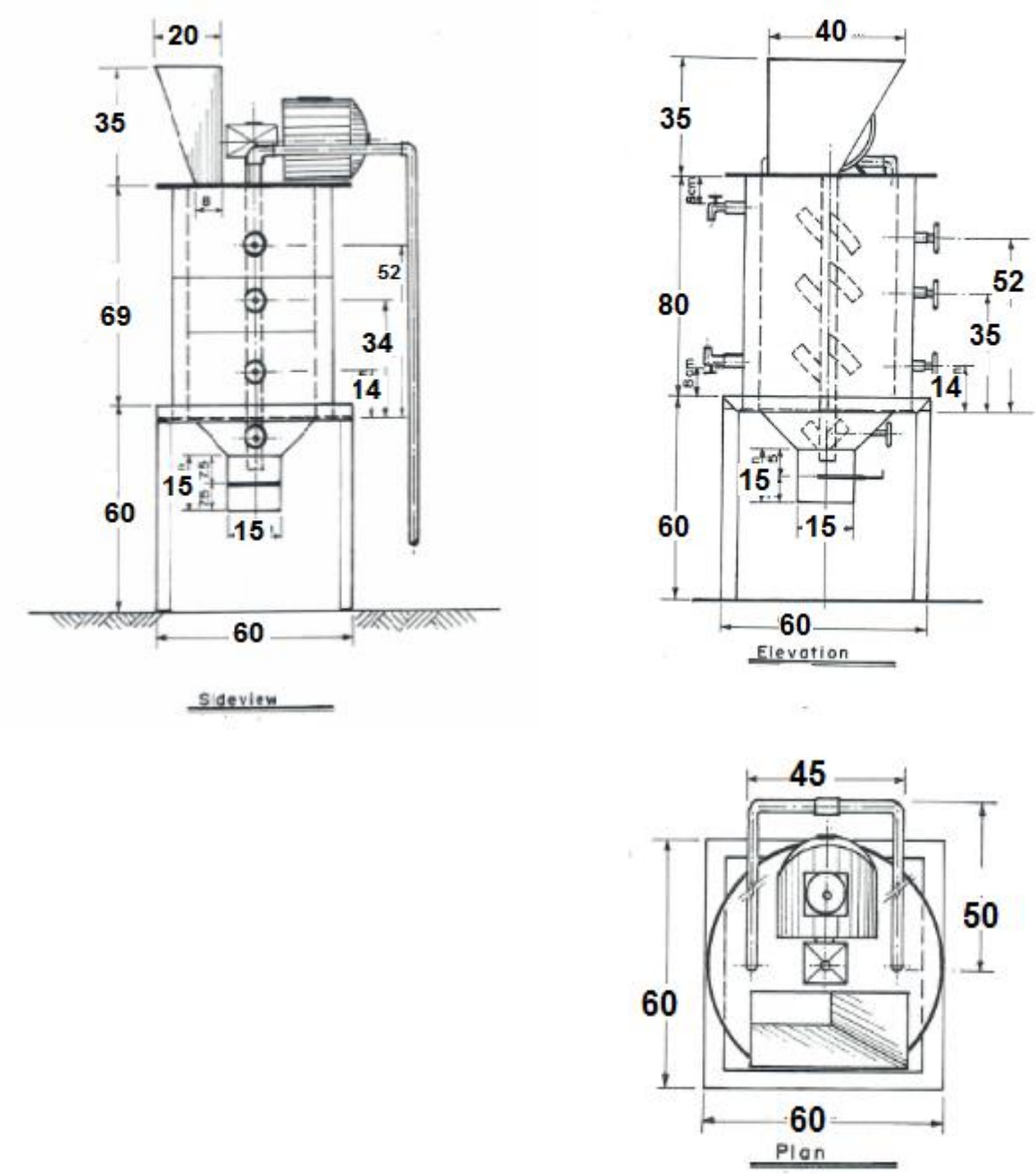
## 2. MATERIALS AND METHODS

### 2.1 Model development

The present study aims to develop a simple bioreactor to create optimal conditions which is necessary for accepted final fodder production. The suggested bioreactor model is a vertical cylinder with a paddle mixer mounted on a shaft running along the central axis of the bioreactor and rotate within the drum. The drum is surrounded by a water-jacket to control bioreactor bed temperature. This bioreactor was made from stainless steel to avoid rusting and to be good heat conduction. Its dimensions are 40 cm, 50 cm and 80 cm inner, outer diameter and length respectively. The total volume of the bioreactor is 100 L. the geometric ratio (H/D) of bioreactor model was about 2 according to **Sangsurasak et al. (1997)**. Stirred-drum of bioreactor is developed to allow adequate mixing of the rice straw and as a result medium aeration. Mixing and aeration of the medium have been furnitured by some devices such as paddles, air flowing in both side of bioreactor by two inlet vertical pipes with diameter of 1.27 cm, and excess gas escaping through an outlet valve. Three analog temperature devices were installed through the bioreactor bed and arranged radically with equal distances to measure the bed temperature profile. Mechanical thermostat composed of heater, control lever and probe was used to adapt the bed temperature according to the optimum temperature (35°C). The heater was merged with the water jacket which surrounds the reactor chamber to release heat to the bed chamber until reaching the suitable temperature, the probe was inserted in the space inside the reactor chamber to connect or disconnect the heater. The schematic diagram of stirred-drum bioreactor which manufactured and used in the experiments was illustrated in Figs. (1 and 2).

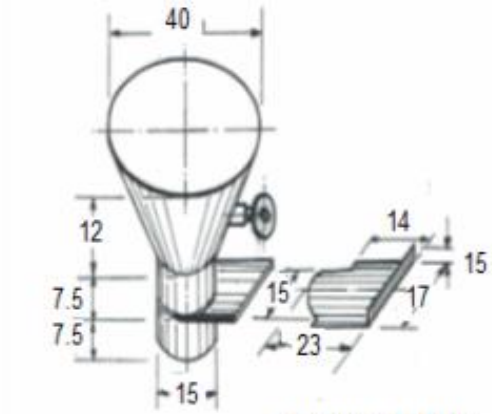
### 2.2 Operational parameters:

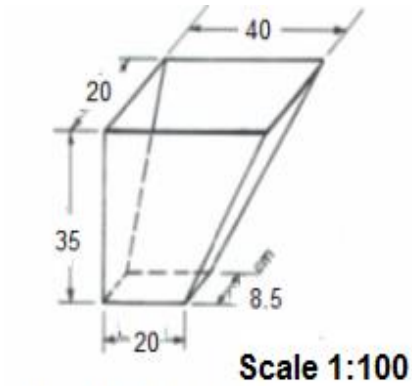
In this study two operational parameters were applied, three levels of Substrate temperatures, $T_1$, $T_2$ and $T_3$ (25, 35 and 45 °C) and three levels of substrate moisture content, $M_1$, $M_2$ and $M_3$ (25, 50 and 65%).

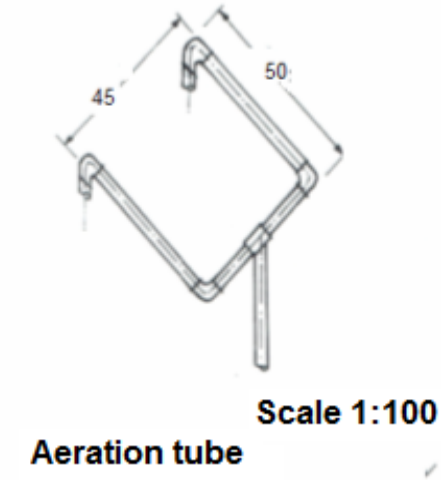


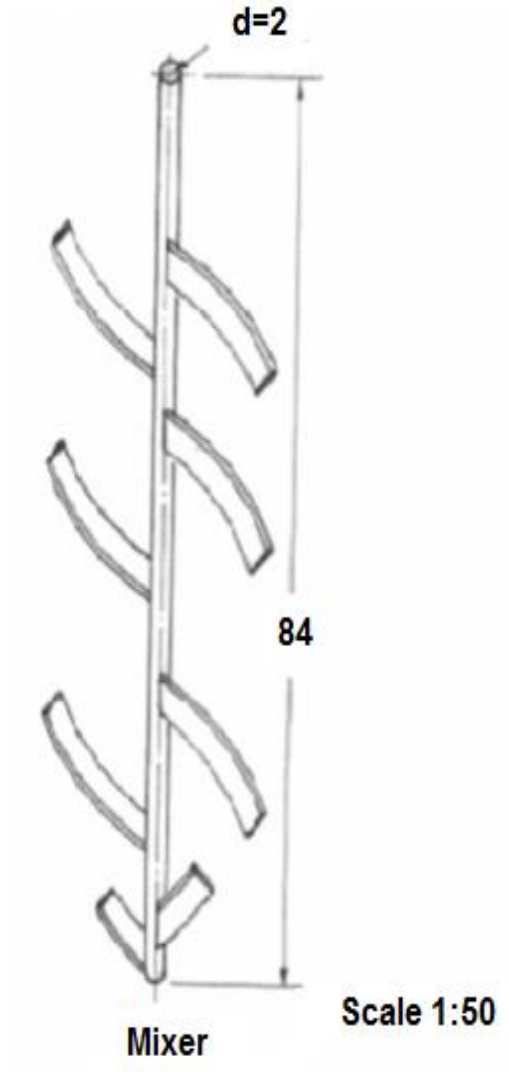

**Scale 1: 100 Dimensions in cm**

**Figure (1): Elevation, side view and plan of the developed bioreactor.**




**Dimensions in cm**

**Figure (2): The internal parts of the developed bioreactor.**

The course of fermentation time was 5 days and the measured parameters which related to microbial growth were Crude protein (CP%) and Crude fiber (CF%). The shaft was rotated 15 min every 24 h before taking samples to remove excess heat and prevent overheating of the system. The aeration rates were natural. Air was flowing in both side of bioreactor by two inlet vertical pipes. The laboratory experiments were carried out in the bioreactor at Testing and Research Station for Tractor and Farm Machinery, Alexandria governorate, Egypt to study the effect of three temperature levels $T_1$ (25°C), $T_2$ (35°C), and $T_3$ (45°C) and three moisture levels $M_1$(25%), $M_2$(50%) and $M_3$(65%) in triplicates for each treatment on fungus growth activity on rice straw under solid state fermentation by ***Trichoderma harzinaum F-418 fungi*** and molasses, valvic solution additive with controlled temperature and moisture at different fermentation time (1, 2, 3, 4, and 5 days) to determined the optimum conditions of fungal treatment in terms of moisture and temperature to compare the chemical analysis of rice straw after fungal treatment Crude protein (CP%), Crude fiber (CF%) with the chemical analysis of rice straw before fungal treatment (control treatment).

**2.3. Materials of study:**

Rice straw Sakha 101 were obtained from Agricultural management kafer El-dawar El-Beheira, governorate, was used as substrate for feeding production, while (***Trichoderma harzinaum F-418 fungi)***, molasses, and valvic solution were obtained from the Biofertilizers Production Unit, Agric. Microbiology. Dept., Soils, Water and Environmental Research Institute. (SWERI) Agric. Res. Center (ARC), Giza, Egypt.

**2.4. Preparation of inocula:**

The microorganism was ***Trichoderma harzianum*** supported from the Biofertilizers Production Unit, Agric. Microbiology. Dept., Soils, Water and Environmental Research Institute. (SWERI) Agric. Res. Center (ARC), Giza, Egypt. ***Trichoderma*** was grown on a potato dextrose agar (PDA) according to **ATCC (1992)** and incubated at 28°C for 48 h. on a rotary shaker. ***Trichoderma*** culture was injected into a sterilized carrier one day before sowing to guarantee the efficiency of rice straw inoculation with the fungi.

**2.5. Preparation of rice straw:**

The rice straw was chopped handly by a small cutter to 2 – 3 cm of length according to recommendations of **Smail et al., (1995)** and treated with ***Trichoderma harzianum F-418 fungi.*** The moisture content of the rice straw was 14% then adjusted to three levels M1(25%), M2(50%) and M3(65%) by spraying solution of water, 4% molasses, 2% valvic and ***Trichoderma harzianum F-418 fungi*** by handy machine gun according quantity of the solutions to be added according to the following equation of **Bart-Plange et al. (2012)** and mixed well and left For 24 hours then

$$Q = \frac{W_i(M_f - M_i)}{100 - M_f}$$

where:

$Q$ Is the mass of solutions to be added in kg

$W_i$ is the initail mass of the sample in kg

$M_i$ is the initial moisture content of the sample in % db and

$M_f$ is the final moisture content in %db

the moisture content of the sample at intervals was measured by a hay moisture meter until proven moisture content of the sample by the ratio required adding. The treated chopped straw well mixed with specific fungal prepared culture at 20% by weight and left for 5 days into bioreactor 100 L capacity. The moisture was chosen according to the studies of **Abdel-Azim *et al*. (2011) and El-Ashry *et al*. (2002)** and each experiment was repeated three times, which leads to 135 experiments for measure the crude protein CP% and crude fiber CF% in this experiment to determined the optimum conditions of fungal treatment in terms of moisture and temperature.

**2.5. Chemical analysis:**

The Proximate chemical analysis supported from the Soil Salinity and Alkalinity Laboratory, Soils, Water and Environmental Research Institute (SWERI), The Proximate chemical analysis of raw material (rice straw

before treatment) and rice straw after treatment and concentrate samples were carried out to determine Crude protein (CP%), Crude fiber (CF%), according to the Association of Official Analytical Chemists **AOAC (1995).**

**1 - Crude protein (CP%)**

Total nitrogen was determined by Kjeldahl method. To estimate the Crude protein (CP%), the measured total nitrogen was multiplied by factor 6.25.

**2 - Crude fiber (CF%)**

The sample, 2 g, was boiled with 200 ml of 1.25% $H_2SO_4$ in 600 ml beaker for 30 min. The solution was filtered through Buchner and boiled in 200 ml 1.25 NaOH for 30 min. The sample was filter again and washed with 75 ml boiled H2O and 25 ml 95% alcohol. The residual was transferred to the crucible and dried at 130°C for 2 h. Cooled in the desicator and weighted. Ignited 30 min at 600°C cooled again in the desicator and weighted. Percent of fiber is calculated by the difference of the sample residue weight before and after the ignition divided by the sample weight **(AOAC, 1995).**

**2.6. Statistical analysis:**

The studied experiments were carried out as factorial experiments in Randomized Complete Block Design (RCPD) in three replications. Least Significant Differences at 0.05 probability level (LSD 0.05) was calculated to compare the differences between treatment means according to **Snedecor and Cochran (1971).**

## 3. RESULTS AND DISSCUSSION

**3. 1. Crude protein content" CP" (%)**

The crude protein content (CP%) of rice straw before fungal treatment (control treatment) was 2.90%. The effect of fermentation time (days) on crude protein content CP (%) at three temperature levels ($T_1$,$T_2$ and $T_3$ ºC) and three moisture levels ($M_1$, $M_2$, $M_3$ %) for treated rice straw with ***Trichoderma harzinaum F-418 (TH)*** are shown in Fig. (3).The statistical analysis of crude protein content (CP %) data showed that it was significant effect by temperature, moisture and fermentation time. Also, it can be noticed that, crude protein content CP% increases with the

increase of fermentation time until 4 and 5 days for all temperature and moisture levels under study. Crude protein values were 5.10, 5.45 and

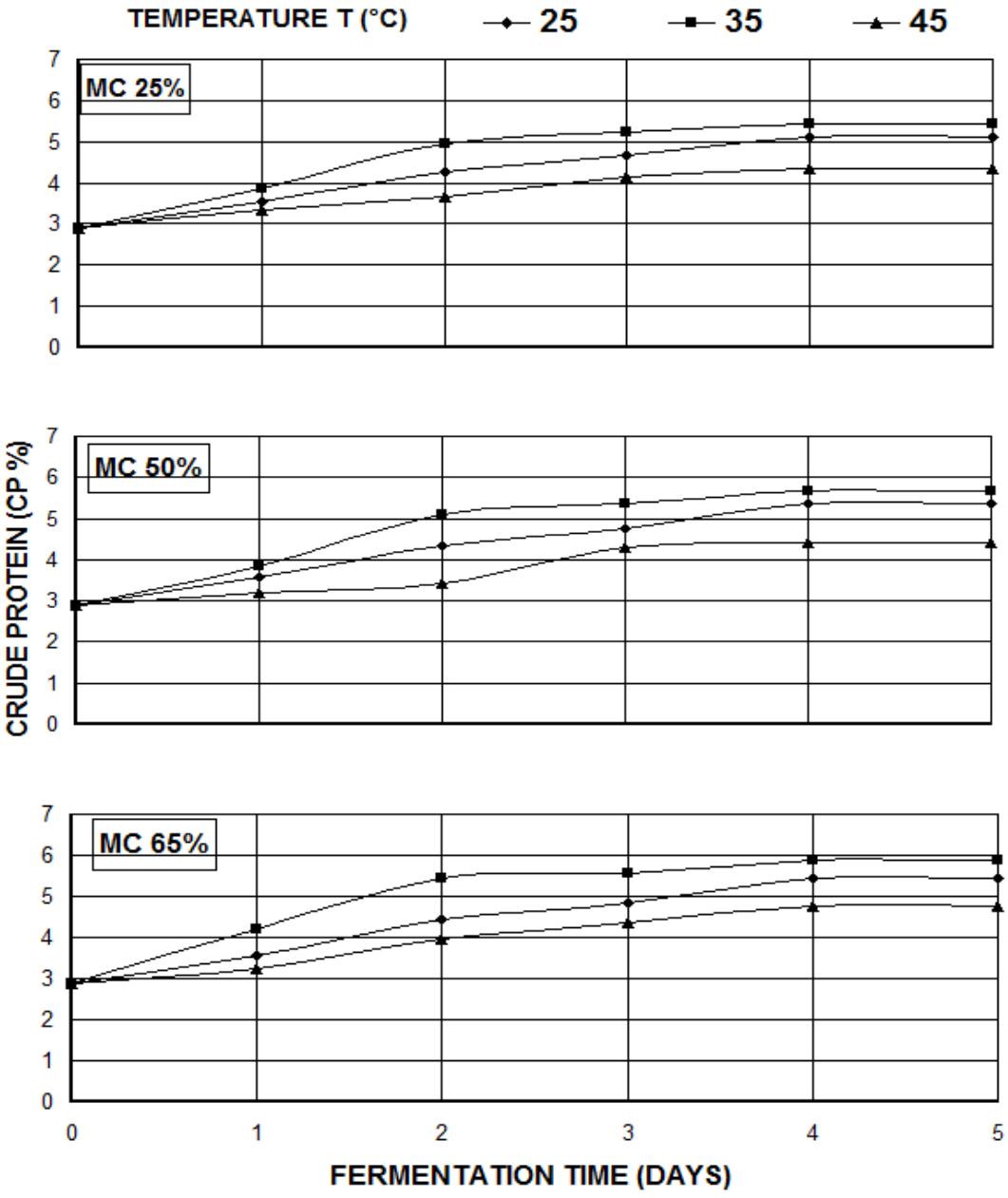



Figure (3): The effect of fermentation time (days) on crude protein content CP (%) at different temperature and moisture levels.

4.33 for $T_1$, $T_2$ and $T_3$ respectively at moisture content $M_1$(25%) after 4 and 5 days. While these values were 5.35, 5.65 and 5.35 for $T_1$, $T_2$ and $T_3$ respectively at moisture content $M_2$(50%). At $M_3$ (65%) the crude protein content values were 5.45, 5.88 and 4.78 after 4 and 5 days for $T_1$, $T_2$ and $T_3$ respectively. It is obvious that the best value of crude protein content was 5.88 % obtained from the treatment $T_2M_3$ by increasing 103 % of control treatment. While treatment $T_2M_2$ gave 5.65% of crude protein content by increasing 95 %. At treatment $T_2M_1$, it was 5.45% by increasing 88%. This may be due to the capture of excess nitrogen by aerobic microbes and conversion of the same into microbial protein during solid-state fermentation. Generally, the biological treatment with ***Trichoderma harzinaum F-418 fungi (TH)*** was led to crude protein augmentation and reduces the crude fiber. Similar results were reported by **Langer et al. (1980)**. This may be because the bioreactor was designed to allow adequate aeration and mixing of the substrate. Mixing and aeration have been explored by rotating paddles mounted on a shaft along the central axis. The growth of the inoculums in bioreactor is considered to be better and more uniform. Also water jacket around bioreactor removed the excess heat.

**3.1.2. Crude fiber content" CF" (%)**

Fig. (4) shows the effect of fermentation time (days) on crude fiber content CF% at three temperature levels ($T_1$,$T_2$ and $T_3$ ºC) a and three moisture levels ($M_1$, $M_2$, $M_3$ %) for treated rice straw with ***Trichoderma harzinaum F-418 (TH).*** The crude fiber content CF% of rice straw before fungal treatment (control treatment) was 40.30%. Results indicate that the crude fiber content decreased with the increased of fermentation time until 4 and 5 days. The crude fiber content values at the moisture level (M1) and the temperature levels $T_1$, $T_2$ and $T_3$ were 31.92, 28.55 and 30.85 respectively. At the moisture level ($M_2$), the crude fiber values were 33.10, 29.08 and 32.25 at temperature levels $T_1$, $T_2$ and $T_3$ respectively. While they were 30.97, 28.35 and 30.25 at the moisture level ($M_3$) and temperature levels $T_1$, $T_2$ and $T_3$ respectively. The statistical analysis of data showed that the crude fiber content was significantly affected by temperature, moisture and fermentation time. The crude fiber data showed

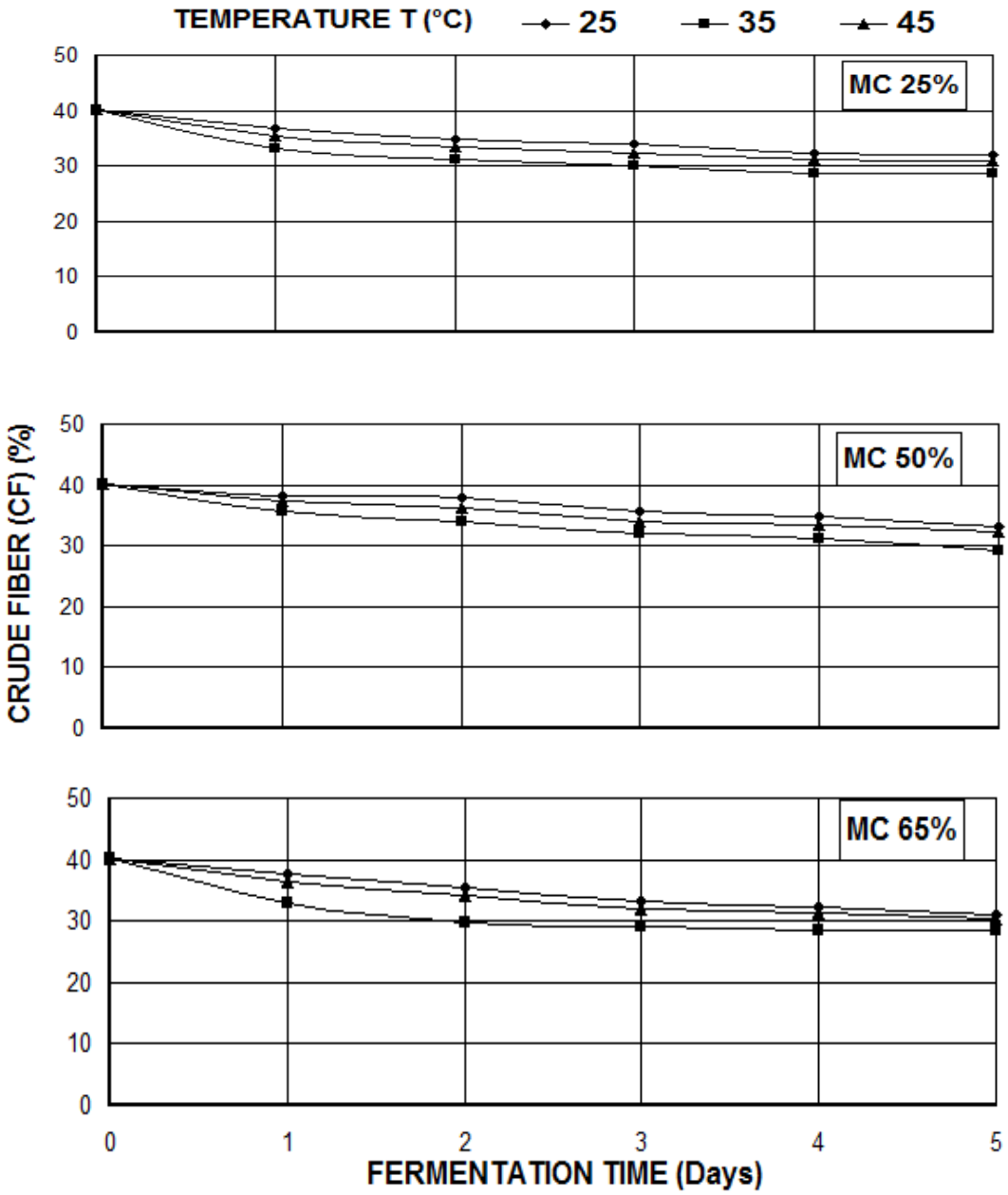



Figure (4): The effect of fermentation time (days) on crude Fiber content CF (%) at different temperature and moisture levels.

that the treatment of $T_2M_3$ and fermentation time of 4 and 5 days gave the best value of crude fiber content where it was 28.35 % by decreasing of 30% compared to the rice straw before fungal treatment (control

treatment). Also, the treatments $T_2M_1$ and $T_2M_2$ showed decreasing of 29 % and 28 % compared to the control treatment respectively. This may be due to the capture of excess nitrogen by aerobic microbes and conversion of the same into microbial protein during solid-state fermentation indicating their influence on hemicelluloses breakdown as the effect of the biological treatment These observations were agreement with **Kholif (2005) and Mahrous (2005)**. Generally, the biological treatment with TH led to increase crude protein and reduce the crude fiber (similar results were reported by **Langer et al. 1980**). This may be due to the design of the bioreactor which allow to adequate aeration and mixing of the substrate. (Mixing and aeration have been explored by rotating paddles mounted on a shaft along the central axis.The growth of the inoculums in bioreactor is considered to be better and more uniform. Also water jacket around bioreactor removed the excess heat).

## 5.CONCLUSION

In this research, a development of bioreactor was made to enrich the protein of rice straw using solid-state fermentation. The fermentation time was 5 days and parameters related to microbial growth crude protein content % (CP%), and crude fiber content % (CF%) were measured every 24 hr. The highest crude protein and the lowest crude fiber values were obtained at the temperature of 35 °C and the moisture content of 65% after 4 and 5 days. The best value of crude protein was 5.88% which increased 103% of the control treatment and the best value of crude fiber content was 28.35% wich decreased 30% of control treatment. It is evident that these values demonstrated good operation of fermentation in bioreactor.

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**الملخص العربى**

## تطوير مفاعل حيوي لإثراء بروتين المخلفات الزراعية

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تهدف هذه الدراسة إلى تعظيم استخدام المخلفات الزراعية وذلك بتصنيع وتطوير مخمر حيوي واستخدامه في إنتاج أعلاف غير تقليدية للحيوانات من المخلفات الزراعية وخاصة قش الأرز عن طريق المعاملة البيولوجية الهوائية للمخلفات الزراعية باستخدام بعض الكائنات الحية الدقيقة ( الفطريات المحللة للسيليلوز ) تحت الظروف الهوائية لإحداث تغيرات طبيعية وكيميائية في مكونات المخلفات الزراعية بهدف تحسين استساغتها وقيمتها الغذائية. في هذه الدراسة تم دراسة تأثير ثلاث مستويات لدرجة الحرارة $T_1$ (25°C)، $T_2$ (35°C) و$T_3$ (45°C) وثلاث مستويات للرطوبة $M_1$ (25%) ، $M_2$ (50%) و$M_3$ (65%) وفترات تخمر لمدة ٥ أيام وتم دراسة تأثير هذه العوامل على كل من نسبة البروتين الخام ونسبة الألياف الخام فى عينات قش الأرز المعالج بالفطر ومقارنتة بنسبة البروتين الخام ونسبة الألياف الخام فى عينات قش الأرز الغير المعالج، مع ثبوت كل من حجم العينة (١ كجم) وفترة التقليب اثناء فترة التشغيل بمعدل ١٥ دقيقة كل ٢٤ ساعة عند أخذ العينة بمعدل ٥٠ لفة /دقيقة وكان مرور الهواء طبيعيا بدون دفع هواء من خلال انبوبتين بها ثقوب وقد تم تقييم أداء المفاعل باستخدام معيارين هما البروتين الخام والألياف الخام بالعينة خلال فترة التخمير وهى ٥ أيام. ويمكن تلخيص النتائج المتحصل عليها فيما يلى: كانت قيمة كل من البروتين الخام والألياف الخام فى معاملة الكنترول ٢,٩% و ٤٠,٣٠% على الترتيب. وكانت أعلى قيمة لمحتوى البروتين الخام هي ٨٨و٥ % للمعاملة $T_2M_3$ بعد ٤ و٥ أيام من التخمير بنسبة زيادة تمثل ١٠٣ % عن معاملة الكنترول ، بينما كانت أقل قيمة لمحتوى الألياف الخام هى ٣٥و٢٨ % للمعاملة $T_2M_3$ بنسبة تقل عن معاملة الكنترول بمقدار ٣٠ % بعد ٤ و٥ أيام من التخمير ومن المعروف أنه كلما زادت نسبة البروتين وقلت نسبة الألياف فى المعاملة دلت على جودة القيمة الغذائية بها وأن عملية التخمير فى المفاعل كانت جيدة.

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