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Bioconversion of Saw Dust Powder Acid Hydrolysis to Single Cell Protein by the Yeast *Candida tropicalis*

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Abstract

This study was aimed to grow the local isolates yeast *Candida tropicalis* on locally prepared sawdust powder hydrolysate as a basal medium and carbon source with respect to single cell protein (SCP) production. The saw dust powder was treated with 10% H_2SO_4 for one hour at 100 ^{0}C. After cooling the supernatant containing the isolated sugar separated from debris by filtration and used as a carbon source and basal medium for yeast growth and SCP production. A high amount of SCP was achieved after five days of incubation, the percentage of the produced SCP 39.05% of the biomass dry weight, which is equivalent to 3.07g/l. SCP formation biosynthesis was affected by the level of nitrogen present in the medium; a high amount of protein being achieved in fermentation medium containing 0.3% urea in which the percentage of the yielded SCP was increased to 42.54%, (3.71g/l) of the biomass dry weight. The effect of varying nitrogen sources on SCP accumulation was also assayed. Medium containing ammonium phosphate greatly stimulated protein production in which the produced SCP was increased to reach 5.90 g/l. The produced amount equivalent to (48.22%) of the biomass dry weight. Conversely, a medium containing sodium nitrate had a suppressive effect on SCP production. This evidence clearly suggests that the metabolic versatility of *Candida tropicalis* may be employed in the conversion of low-grade material into high SCP product.

Keywords: *Single cell protein, Saw dust hydrolysis, Nitrogen sources, Candida tropicalis.*

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I. INTRODUCTION

Microorganisms have been employed for many years in the production of high protein foods such as cheese and fermented soybean products (Nasseri *et al*., 2011). Since a large proportion of cell dry weight is accounted for protein, the nutritional value of a microbial-derived food source is determined by the levels of protein produced (Pape, 2002). The use of microbes in the food industry has several advantages over the use of animals. Firstly, the growth of microbes is much faster than animals making them more suitable for food in a short time. Secondly, a broader range of substrates and nutritional supplements, for microbes are available with most being cost-effective.

The development of microbial systems for the food industry has Firstly, growth of microorganism is very much faster than of animals. Secondly, a broader range of materials may be considered as suitable substrates depending on the microorganism chosen. The two chief strategies with regard to the substrate to consider low-grade waste material or to use a relatively simple carbohydrate source to produce microbial material containing very high quality of protein (Reed and Nagodawithana, 1995; Carimella *et al*., 2017). Moreover, (SCP) amino acid composition of microbial SCP has good nutritional value because of it is high content of essential amino acid (Adedayo *et al*., 2011), organic acids, vitamins, fat and minerals (Asad *et al*., 2000; Jamel *et al*., 2008).

Microorganisms have the ability to upgrade low protein organic material to high protein food, and this has exploited by industry. This phenomenon was employed in Germany during the first world war when the growth of Saccharomyces cerevisiae was exploited for human consumption. Moreover, *Candida arborea* and *Candida utilis* were used during the second world war and about 60% of the country prewar food input was replaced (Litchfield, 1983; Arora *et al*., 1991).

The use of natural cheap substrates and waste industrial products for cultivating microorganisms appear to be a general trend in studies of applied nature (Grewal *et al*., 1990; Osho, 1995; Haider *et al*., 1989).

Various natural products have been used for the production of microbial protein. For instance, Kuzmanova *et al*., (1989) have used grape juice byproduct as a carbon source. While Haider and El-hassy (2000) have used date extract supplemented with nitrogen source as a substrate, while, Osho (1995) has used cashew apple juice. Moreover, waste potato together with corn cob (Al-Hadithi *et al*., 2018) and date syrup waste (Al-Faris *et al*., 2019) have been used as cheap substrates and as a carbon source for SCP production.

Several investigations were carried out using cellulose and hemicelluloses waste as a suitable substrate for increasing SCP production (Azzam, 1992; Pessoa *et al*., 1997; Zubi,

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2005). Moreover, a number of industrial waste products are being used for the production of SCP (Abou-Hamed, 1993; Nigam, 2000; Abood *et al*., 2017; Spalvins *et al*., 2018). The raw material was hydrolyzed by diluted acid or base and then supplemented with some nitrogen and phosphorous salts. Besides that photosynthetic purple non-sulphur bacteria (*Rhodopseudomonas palustris*) were also used for the same purposes (Carimella *et al*., 2017).

The objective of this study was to investigate the ability of locally isolated Candida tropicalis to utilize saw dust powder hydrolysate as basal substrate and carbon source with the addition of nitrogen concentrations and sources to determinate the levels of produced microbial SCP.

II. Materials and Methods

A. The microorganism

Yeast Candida tropicalis locally isolated from a swimming pool in Duhok province and identified by Abdullah (2015) was used throughout this investigation. It was maintained on potato dextrose agar (PDA) slants and sub-cultured for activation at intervals from 4-5 weeks.

B. The production of inoculum

The malt-yeast extract (MY) broth medium (glucose, 1.0, peptone, 0.5; yeast extract, 0.3 and malt extract 0.3) % was used for the preparation of the inoculum in which the pH of 250ml conical flask containing 50 ml of MY was adjusted to 5.6 before autoclaving for 15 min at 121^0C. After cooling the medium was inoculated from 5 days old yeast slant and incubated in a shaking incubator at 150RPM and 30 ± 1^0C for 3 days. This yeast culture was used for inoculation of each medium used through this study (Haider, 2020).

C. Sawdust powder acid hydrolysis medium

The medium was prepared as explained earlier by (Abdulazeez and Haider, 2020). The sugar concentration of the acid hydrolyzed sawdust was measured spectrophotometrically at 490nm using Jenway 6305 spectrophotometer, U.K) to prepare basal medium containing 10% carbon source in the form of saw dust hydrolysis for growth of the yeast and production of SCP.

D. Experiments

1. Different incubation periods (2, 3, 4, 5and 6) days were tested
2. Different levels of nitrogen (0.1, 0.2, 0.3, 0.4 and 0.5) % in the form of urea were used.
3. Different nitrogen sources, namely, urea, NH4Cl, $(NH_4)_2SO_4$, NaNO3, $(NH_4)_2HPO_4$ and peptone were used containing the same level of nitrogen present in 0.3g urea in 100ml of the medium.

E. Cultural condition

The fermentation media were distributed into 250ml conical flasks in triplicate samples receiving every 49 ml of broth medium and the pH was adjusted to 5.6 (Haider *et al*., 1989). Afterwards, the flasks were plugged and covered by aluminium foil before being autoclaved for 15min at 121^0C. After cooling, the culture flasks were inoculated with 2% yeast cells suspension. The inoculation culture flasks were incubated for sufficient time in a rotary incubator (150 RPM) at 30°C and at designated intervals, three replicates of each treatment were withdrawn for further analysis.

F. Analytical methods

The initial and final pH of the culture media were adjusted using a hydrogen ion concentration instrument (pH METER SCHOTT TYPE (G842). Biomass yeast dry weight was determined by centrifugation of the fermentation media and isolation of cell debris as previously described (Haider, *et al*., 1989).

The described method of Dubois *et al*., (1956) was applied to determine the initial and residual sugar and total protein content in dried yeast cells was assayed by the method of Legatt-Bailey (1967). The data was statistically analysed using one-way ANOVA and LSD tests.

III. Results

A. Effect of incubation periods

This experiment was designed to determine the best period of incubation that stimulate single cell production by the yeast *Candida tropicalis*. The present data clearly indicate that the five days of incubation was optimal with SCP production reaching 7.86g/l and 3.07g/l in respect to yeast growth and SCP formation, respectively (Table 1).

Table 1. Effect of incubation periods on SCP production by *Candida tropicalis*[*]

Days of incubation	Dry weight (g/l)	SCP g/l	SCP %	Residual sugar %	Final pH
2	4.3 (0.01)	1.54 (0.05)	33.85	0.10 (0.014)	5.31 (0.018)
3	6.98 (0.00)	2.50 (0.03)	35.86	0.083 (0.006)	5.22 (0.004)
4	7.13 (0.06)	2.58 (0.009)	36.31	0.070 (0.003)	5.14 (0.21)
5	7.86 (0.01)	3.07 (0.002)	39.05	0.054 (0.051)	5.03 (0.06)
6	7.42 (0.08)	2.73 (0.007)	36.81	0.048 (0.005)	5.02 (1.002)

[*]Each number represent the mean of three replicates and the number between brackets represent (S.D.)

B. Effect of nitrogen Level

The nitrogen content of the medium was altered by varying the concentration of urea added to the saw dust hydrolysate fermentation medium. The organism was incubated for five days (Table 2).

Table 2. Effect of urea concentration on SCP production by *Candida tropicalis*[*]

Urea %	Dry weight g/l	SCP g/l	SCP %	Residual sugar %	Final pH
0.1	7.23 (0.005)	2.53 (0.061)	34.99	0.087 (0.092)	5.3 (0.00)

0.2	8.01 (0.025)	3.04 (0.041)	37.95	0.058 (0.00)	5.18 (0.001)
0.3	8.72 (0.003)	3.71 (0.003)	42.54	0.049 (0.00)	5.10 (0.00)
0.4	8.32 (0.101)	3.31 (0.005)	39.78	0.058 (0.040)	5.00 (0.001)
0.5	7.91 (0.003)	2.96 (0.100)	37.42	0.063 (0.00)	4.95 (0.004)

*Each number represent the mean of three replicates and the number between brackets represent (S.D.).

C. *Effect of nitrogen sources*

As demonstrated in Table 3, nitrogen content in the media is of extreme importance to protein yield. For this reason, it was investigated which nitrogen source would be of most value in this experiment. The organism showed different responses toward different sources of nitrogen used and such results are supported when the protein formation is calculated as a percentage of dry yeast cells. The medium containing ammonium phosphate as nitrogen highly stimulated SCP production to reach 5.90g/l which is equivalent to (48.22%) of the biomass dry weight, followed by medium containing urea as a nitrogen source and the produced amount is 3.66g/l, (42.14%). Conversely, a medium containing ammonium chloride greatly reduced protein synthesis (1.47g/l, 28.22%). Statistical analysis showed that the increased effect on SCP production by ammonium phosphate was highly significant at a level (α=0.05).

Table 3. Effect of nitrogen sources on SCP production by *Candida tropicalis*[*]

Nitrogen source %	Dry weight g/l	SCP g/l	SCP %	Residual sugar %	Final pH
Urea	8.68 (0.001)	3.66 (0.08)	42.14	0.049 (0.00)	5.0 (0.08)
NH_4Cl	5,24 (0.008)	1.47 (0.00)	28.22	0.052 (0.00)	4.32 (0.15)
$(NH_4)_2SO_4$	7.14 (0.041)	2.59 (0.008)	36.34	0.058 (0.017)	4.35 (0.014)
$NaNO_3$	5.38 (0.71)	1.63 (0.00)	35.89	0.67 (0.001)	4.90 (0.03)
$(NH_4)_2HPO_4$	12..24 (0.081)	5.90 (0.003)	48.22	0.030 (0.003)	5.8 (0.04)

*Each number represent the mean of three replicates and the number between brackets represent (S.D.)

IV. Discussion

The best period of incubation that stimulates single cell production by the yeast *Candida tropicalis* was the five days of incubation with SCP production reaching 7.86g/l and 3.07g/l with respect to yeast growth and SCP formation, respectively. This observation was confirmed further when total SCP formation was calculated as a percentage of dry yeast cells (39.05%). Incubation above 5 days both yeast growth and SCP production slightly inhibited due to many factors such as exhaustion of carbon and nitrogen sources, depletion in the concentration of oxygen, change in pH value of the fermentation and production of a certain toxic metabolite. Similar results have been previously described by Hongpjattarakere and Hkittikun (1995) when they have grown the yeast *Shawnniomyces castellii* on the cassava starch as a carbon source.

Bozakouk (2002) and Zubi, (2005) have found that the optimal periods of incubation for higher protein accumulation by the yeast *C. utilis* and *Fusarium graminearum* was six days. The discrepancy between these results and others with respect to the incubation period mainly depends upon the type of microorganisms and substrate used as a basal medium and carbon source.

The statistical analysis program one-way ANOVA test at the significance level (α=0.05) showed that there is a significant difference within treatments of SCP production. Significance LSD test revealed a significant difference in SCP formation at different incubation periods except for 3 and 4 days treatments.

It is evident as expected, that protein formation is affected by the concentration of nitrogen present in the media. The highest SCP production when calculated as g/l or as a percentage of dry yeast cells was achieved at a concentration of nitrogen present in 0.3% urea in which the total amount of produced SCP was increased to 3.71g/l, (42.54%). The superiority of 0.3% of used urea compared to other concentration is dependent on the N/C ratio in which at this ratio (1/33, N/C) was the best for high yeast growth and SCP accumulation. Gradual increasing of nitrogen source more than 0.3% has inhibition effect in SCP accumulation due to the change of the optimum N/C ratio. This finding agreed with those reported by Haider *et al*., (1989), El-Mashhadany (1995), El-jebory (1997) and Zubi (2005). These results are confirmed when they are statistically analysed at significance (α=0.05). LSD test also revealed that there is a significant difference in the case of SCP production in all treatments.

The medium containing ammonium phosphate as nitrogen highly stimulated SCP production. The stimulating effect of ammonium phosphate is mainly due to adjustment of buffer by retaining the hydrogen ion concentration of (pH) of the fermentation medium at it's optimal condition which stimulates the growth of the yeast and anabolic reactions for biosynthesis of the SCP.

Medium containing ammonium chloride greatly reduced protein synthesis. Similar results have been explained by Haider and El-drossy (2000) when they used date extract as a carbon source and different nitrogen sources for SCP production by the fungus *Fusarium solani*. The inhibitory effect of ammonium chloride is due to the reaction between chloride and hydrogen lead to the formation of hydrogen chloride which reduces the pH of the fermentation medium to level directly affected the yeast growth and anabolic reaction for the biosynthesis of SCP.

On the other hand, Bozakouk, (2002), Zubi, (2005) and Al-Faris *et al*., (2019) have found that ammonium sulphate was superior to other nitrogen sources for SCP production by *Candida utilis*, *Fusarium graminearum* and *Fusarium oryzae* respectively due to the supply the sulphur element

for stimulating the building up of the amino acid containing sulphur such methionine. Moreover, Fatemeh *et al*., (2019) and Obaeda, (2021) explained that SCP accumulation by any tested microorganism was highly affected by nitrogen source added to the fermentation media.

V. Conclusions

This paper clearly demonstrated that maximum SCP production is achieved after five days of incubation when saw dust hydrolysate was used as a carbon source. Accordingly, it can be proposed that the locally isolate *Candida tropicalis* has the ability to upgrade low protein material to higher protein value.

The individual low protein substrate (saw dust hydrolysate) was unable to support high SCP formation without the addition of a certain nitrogen source. The results of the present work also indicate that protein production by the yeast Candida tropicalis was also affected by the level and source of nitrogen added to the medium.

Finally, with the onset of genetic manipulation, it has now become feasible to produce high protein yields by a genetically modified gene in microorganisms. This was explained by Haider *et al*., (1989).

The construction of a gene resulted in a proline enriched protein being expressed in *E. coli*. Thus, by employing such a technique, it will be possible to increase the efficiency of microorganisms to produce a very high SCP yield from low-level substrates

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