KOR. JOUR. MICROBIOL. Vol.12, 31~36(1974)

팔프 및 제지공장 폐수의 처리에 관한 미생물학적 연구(제2보)

—우수균주에 의한 폐수처리 방법에 관한 연구—

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Microbiological Studies on the Treatment of Waste Liquer from Pulp and Paper Industries(II)

—Studies on the Treatment of Waste Liquor with Yeasts—

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ABSTRACT

Four strains of yeasts were chosen from those isolated previously, and a strain from 160 isolates collected in this year were examined for the treatment of pulp waste liquor. Experiments about optimum nutrient condition, composition of cells, and reduction of B.O.D. on the "S" pulp industry waste liquor were performed with 5 strains.

1. The isolates(strain 112) was identified as *Candida utilis*.
2. The optium concentration of 4 components of nutrients were $(NH_4)_2SO_4$ 1g/*l*, yeast extract 70mg/*l*, KH_2PO_4 300mg/*l*, and $MgSO_4 \cdot 7H_2O$ 500mg/*l*.
3. Specific growth ratio of *Candida utilis* KYRI 112 was 0.48/hr at optimum nutrient media and the yield was 0.45%(V/V).
4. *Endomycopsis capsularis* KYRI 613 contained more crude protein than the most of commercial yeasts.
5. The B.O.D. of waste liquor was reduced to 20% of its value by the culture.

INTRODUCTION

Nowadays supply of good protein has been emphasized in its importance and water pollution has been to be a social problem. These two subjects, pollution and production of protein, have been supposed to get a satisfactory solution by microbiological treatments of contaminated waste liquor from various kinds of industries. In this connection preliminary basic research works had been carried out and its results were published in 1971.

Since then yeast strains were newly isolated and stocked continuously and now the experiments have been made in a progressive advance.

MATERIALS AND METHODS

1. Inocula

Five strains of wild yeasts have been used on the physiological experiments.

Four strains have been saved since 1971, but 1 strain was chosen out of 160 strains for its good growth in waste liquor, which were isolated from soil at Mt. Chunma on May, 1973. The unfamiliar

strain was acclimatized with incubating the fresh inocula on the malt extract media or glucose nutrient agar(GNA) media at 30±1°C for 36 hours 2—3 times.

2. Media

The composition of GNA media for acclimatizing the fresh inocula is glucose 20g, peptone 3.5g, yeast extract 5g, KH_2PO_4 2g, $MgSO_4 \cdot 7H_2O$ 2g in 1000m*l* D.W. For the most part effluent samples were neutralized by adding acid solution, and autoclaved for 15 minutes at 15 lbs after filtration.

3. Cultures

Acclimatized inocula were incubated in 500m*l* Erlenmyer flask each containing 100m*l* waste liquor. The cultures were grown on a shaking incubator(130 strokes/min) at 30±1°C. For measuring the degree of growth cell numbers of each culture were counted with a haemacytometer at intervals of 4 hours. Cell volumes were measured after centrifuging for 7 minutes at 3500rpm.

4. Analytical method

Chemical composition of organisms was analyzed by the standard AOAC method.

And determination of the biological oxygen demand(B.O.D.) was based on the standard procedures.

5. Identification

Identification procedures were followed after those recommended by Lodder and Kreger-van Rij(1970).

RESULTS AND DISCUSSION

1. Morphological and physiological properties

The strain 112 was as follows:

1) After growth for 3 days at 25°C shapes of cells were ovoid to long ovoid, and their sizes were 4—6×6—16μ. The streaks were creamy and smooth on the agar media.

2) Pseudomycellia were observed with the Dalmau plate cultures on corn meal agar media, but ascospores and ballistospores were not observed on any media.

2. Characteristics of utilization of carbon compounds

1) Fermentation of carbohydrates:

Glucose+, galactose—, sucrose+, maltose—, lactose—, raffinose+

2) Assimilation of carbohydrates:

Glucose+, L-rhamnose—, galactose—, ethanol+, sucrose+, glycerol+, maltose+, D-mannitol+, lactose—, salicin+, raffinose+, lactic acid+, L-arabinose+, citric acid+, soluble starch—, succinic acid+, D-xylose+, inositol—, D-ribose—

3) The strain 112 assimilated potassium nitrate and grew in vitamin-free media.

The taxonomical properties of the strain agreed with the standard description, but a bit difference was found in arabinose.

Considering its assimilative characteristics of nitrate the strain 112 was supposed to be more similar to *Candida utilis* than *Candida solani* and *Candida guilliermondii*.

4) Because of double role of HNO_3, as a neutralizing agent and as a nitrogen source, neutralization of waste liquor with HNO_3 was supposed to be more useful than H_2SO_4. Indeed neutralization with HNO_3 group increased its productive ratio(A/A_0, A_0:control group) 1.3 folds than H_2SO_4 group.

3. Seeding

It is generally known that both the quality and quantity of seed yeasts are important in obtaining good yields. Acclimatized inocula prepared with malt extract media showed a higher productive

ratio than with GNA media. Its optimum seeding yeast number is 1×10^7 cells/m*l*, whose results agreed well with Mezinsky. Mezinsky indicated that increased amounts of seed yeasts, within certain limits, resulted in higher yields of yeasts than the content of vitamins in the seed yeasts were the factor of prime importance in determining its yeast-forming efficiency.

4. Optimum nutrient concentration

1) Nitrogen source

Nitrogenous material is essential to the continual production of new protoplasm. The yeasts generally derive those elements from such relatively simple substances as ammonium salts, nitrates, amino acids, and amides.

It has been believed that in a well balanced medium ammonium sulfate salts support at least as much growth as any other single source of nitrogen. In this experiment, ammonium sulfate was used for examination of optimum concentration of nitrogen on the growth of strain 112.

The results shown in Fig. 1 indicate that the optimum concentration of ammonium sulfate lies within 1g/*l* and over 1g/*l* the productive ratio was decreased slightly. Productive ratio of strain 112 was 2.3 folds at the optimum concentration.

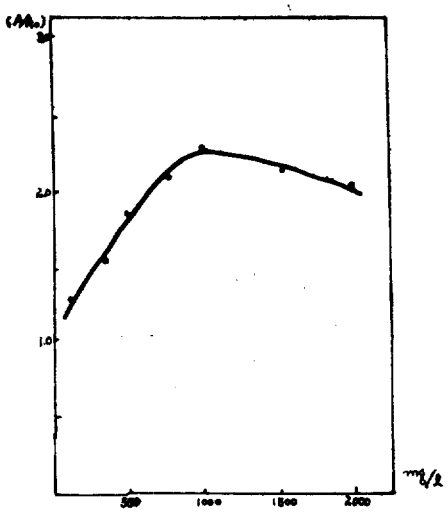

Fig. 1. Effect of $(NH_4)_2SO_4$ concentrations on the growth of *Candida utilis* KYRI 112

2) Source of nutrilite

Few experiments about effect of yeast extract concentration on the growth of yeasts had been practiced in the research works about microbiological treatments of waste liquor from pulp industries. As shown in Fig. 2 optimum concentration was revealed as 70mg/*l* and its productive ratio of strain 112 showed 2.8 folds at the optimum concentration. Growth of yeasts was affected markedly by fluctuation of the concentration below 70mg/*l*.

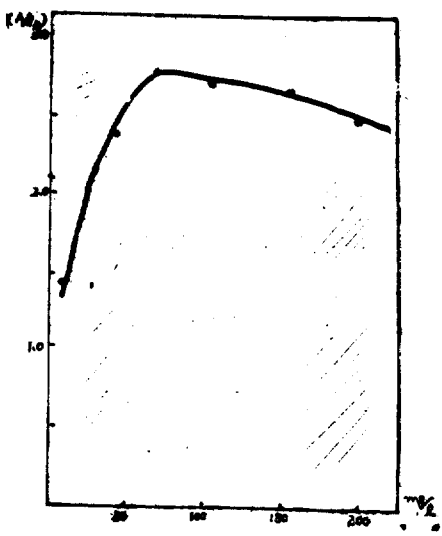

Fig. 2. Effect of yeast extract concentrations on the growth of *Candida utilis* KYRI 112

3) Phosphorus sources

It has been generally known that phosphorus is essential for the growth of yeasts. This element plays an important role in the mechanism of carbohydrate metabolism being concerned with adenosine monephosphate-adenosine diphosphate(A-

MP-ADP) reaction. As shown in Fig. 3, maximum productive ratio was about 2 folds at 300mg/*l*, and neglectly affected as the concentration being increased.

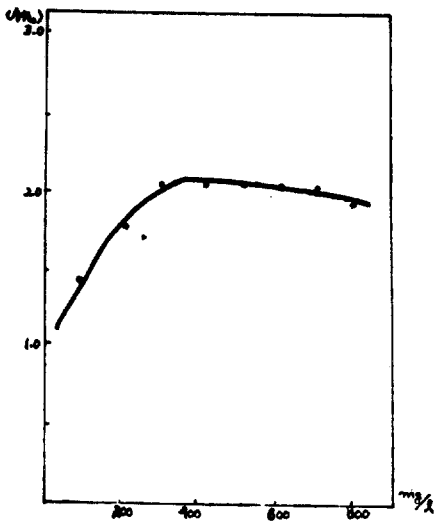

Fig. 3. Effect of KH_2PO_4 concentrations on the growth of *Candida utilis* KYRI 112

4) Magnesium source

Lesh *et al.* indicated that deficiency of magnesium in synthetic media reduced the response of certain strains of *Saccharomyces cerevisiae* as "bios factors".

There can be no doubt that magnesium is essential for the production of higher yields of yeasts.

It was known that magnesium was associated with the metabolism both of carbohydrates in activation of phosphate-transferring enzyme and of nitrogenous compounds. Fig. 4 shows effects on the growth by the fluctuation of magnesium concentrations. In this figure optimum concentration is revealed as 500mg/*l* and its productive ratio is 1.8 folds. This results agreed with Fulmer *et al.* who indicated that optimum concentration lied within 200—400mg/*l* and its productive ratio showed 1.8 folds. Fulmer *et al.*

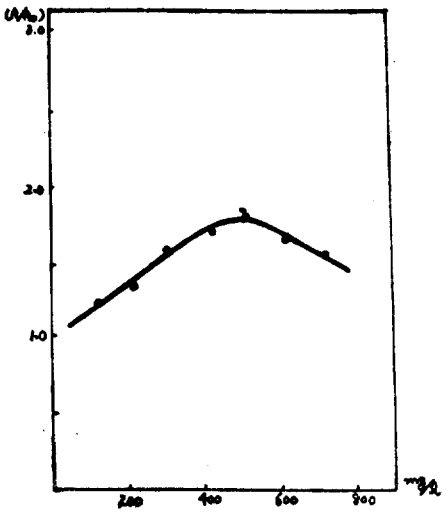

Fig. 4. Effect of $MgSO_4 \cdot 7H_2O$ concentrations on the growth of *Candida utilis* KYRI 112

indicated that growth of yeasts was inhibited in the range of 400—1000ppm slightly.

But the strain 112 was inhibited more markedly in this range. It is supposed that the waste liquor contains sufficient amounts of magnesium and calcium components. With incubation of *Candida utilis* KYRI 112 in the waste liquor containing suitable amounts of 4 compounds, specific growth of this strain was 0.48/hr, and period of adaptation was 2—4 hours, and time to reach the stationary phase was 14—16 hours, and the yield was about 0.45%(V/V). B.O.D. was reduced down to 80% with incubation of the yeast and separation of the yeast from culture.

5. The composition of yeasts

As shown in Table 1, the strain 613 contained much crude protein than *Candida utilis* which was commercially pro-

duced from the spent sulfate liquor.

Therefore this strain is expected to be a good one for commercial products as a fodder yeast. As shown in Table 2 strain 613, compared with the *Saccharomyces cerevisiae*, contained a great deal of aspartic acid, serine, and alanine. But *Saccharomyces cervisiae* contained a trace of these elements.

Table 1. Composition of yeast extract grown on the waste liquor from pulp industry

Component / Strain	Moisture (%)	Crude fat (%)	Crude protein (%)	Ash (%)	Carbohydrate (%)
100	76.47	0.79	9.31	0.72	12.81
235	75.65	0.37	11.37	0.79	11.82
311	76.53	0.32	9.00	1.19	12.96
613	82.65	0.09	10.50	0.50	7.26

Table 2. Amino acid composition of yeast grown on the waste liquor from pulp industry(strain 613).

	Total(mg/%)	Free(mg/%)
Aspartic acid	577,526	24,894
Threonine	312,352	16,204
Serine	426,747	25,774
Glutamic acid	688,591	64,393
Proline	151,273	13,880
Glycine	329,619	32,462
Alanine	495,272	56,001
Cystine	597,769	15,709
Valine	29,118	Traces
Methionine	33,457	〃
Isoleucine	233,565	19,381
Tyrosine	416,085	33,434
Phenylalanine	208,021	14,375
Lysine	234,386	18,741
Histidine	466,341	31,801
Arginine	173,417	3,451
Leucine	394,044	52,667
Total	5,765,583	

摘 要

전보에서 선정한 균주중에서 4균주를 선정하고 금년에 분리한 160균주 중에서 1균주를 선정하여 S팔프공장 폐수에서의 최적배지 조건과 세포의 성분을 분석하고 B.O.D.의 제거도(度) 및 수율을 밝혔다.

1. 선정한 균주(strain 112)는 *Candida utilis*로 동정하였다.
2. $(NH_4)_2SO_4$는 1g/*l*, yeast extract는 70mg/*l*, KH_2PO_4는 300mg/*l*, $MgSO_4 \cdot 7H_2O$는 500mg/*l*에서 가장 높은 수율을 보였다.
3. 최적 배지에서의 비증식 속도는 0.48/hr이었고 이때 수율은 0.45%(V/V)였다.
4. *Endomycopsis capsularis* KYRI 613은 제품화된 사료효모 *Candida utilis* 보다도 높은 단백질을 함유하고 있었다.
5. B.O.D.의 제거도(度)는 약 80%정도였다.

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