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PRODUCTION OF YEAST CELLS FROM HYDROCARBONS

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Seventeen strains of yeasts were propagated on media containing kerosene, n-hexadecane and Sui gas (a natural gas containing 95% methane), as the sole source of carbon. Kerosene inhibited growth of all the microorganisms, whereas n-hexadecane supported it. 5 strains utilised Sui gas, but the growth was less on n-hexadecane. Addition of 0.2% peptone to the medium stimulated the growth. A locally isolated yeast strain No. Y3/WRL gave a maximum (78.9%) conversion of n-hexadecane into cellular mass.

Utilization of hydrocarbons by microorganisms has been studied during the last decade. A large number of bacteria, yeasts and molds, capable of assimilating hydrocarbons, have been reported by various workers.

Yeast propagation on hydrocarbon was first reported by Champagnat *et al.*[1] in 1963. Miller *et al.*[2] isolated a strain of *Candida intermedia*, capable of utilising hydrocarbons, from soil. Later, these workers observed that a mixed culture of *Candida intermedia* and *Candida lipolytica* produced better cell yields on gas–oil fraction.[3,4] Takahashi *et al.*[5] screened a large number of typical and unknown yeast strains for utilisation of hydrocarbons. Dostalek *et al.*[6] studied biomass production and deparaffination of gas oil by *Candida lipolytica*.

Yeast has been found to be more efficient hydrocarbon converter than bacteria. It is also easier to harvest yeast cells from a medium.

Utilisation of kerosene, n-hexadecane and Sui gas (a natural gas containing 95% methane) by various strains of yeasts is reported in this paper.

Experimental

Organisms

Seventeen strains of yeasts belonging to different type culture collections were employed during these investigations.

Media

The following are the two media employed:-

Medium A.—Solution 1: Ammonium nitrate 3.0 g, potassium dihydrogen phosphate 2.5 g, magnesium sulfate 7 Aq. 1.0 g, boron 0.01 ppm, copper 0.01 ppm, iodine 0.1 ppm; iron 0.05 ppm, and zinc 0.07 ppm, distilled water 900 ml.

Solution 2: Inositol 0.20 mg, thiamine HCl 0.04 mg, riboflavin 0.20 mg, pyridine HCl 0.40 mg, nicotinic acid 0.2 mg, *p*-aminobenzoic acid 0.2 mg, calcium pantothenate 0.2 mg, and biotin 0.2 mg prepared the stock solution containing the desired concentration in 65 ml distilled water.

Medium B.—Ammonium sulfate 3.0 g, potassium dihydrogen phosphate 2.5 g, magnesium sulfate 7 Aq. 1.0 g; distilled water 1 liter.

Medium A was used for liquid hydrocarbons, while Medium B was employed for Sui gas.

Solution 1 (18.0 ml) was taken in Erlenmeyer flask of 50-ml capacity and sterilised at 10 lb/in^2 for 15 min. Solution 2 was sterilized by passing through Seitz membrane filters. 1.3 ml of this solution containing the desired concentrations of the vitamins was transferred aseptically into the flasks. The pH was adjusted to 4.5. 0.2-ml portions of n-hexadecane, kerosene, sterilised by filtration, were aseptically added into each flask.

Medium B (19.5 ml) was transferred into 300-ml towers, and sterilised at 10 lb/in^2 for 15 min. H_2S and mercaptans present in Sui gas were eliminated by passing over a column of activated charcoal. The gas was sterilised by passing through glass wool plug and distilled water.

The fermentation was carried out in Erlenmeyer flasks of 300-ml capacity. These flasks were shaken on a rotary shaker (125 rev/min) and were maintained at a temperature of 30 ± 0.5°C.

Preparation of Inoculum

The inoculum was prepared in a 300-ml Erlenmeyer flask to which 50 ml of the medium A, alongwith 1 ml n-hexadecane was added. The flask was incubated at 30°C for 5 days on a rotary shaker. The cells were harvested in a tared centrifuge tube, resuspended in saline water and finally given two washings with sterile distilled water. 0.5 ml of the suspension containing 10–20mg of the cells (on wet basis) was used as inoculum.

Hydrocarbon Utilisation Test

The ability of a strain to assimilate the hydrocarbons was qualitatively determined turbidimetrically. The findings were further confirmed microsocopically. Division of microbial cells was taken as an indication of growth on hydrocarbons.

Measurement of Cell Mass

The cells were centrifugated in a tared centrifuge tube. They were resuspended in 5 ml saline water and recentrifuged. Finally the cells were washed with distilled water. The washed cells were dried at 105°C for determination of dry mass. The percentage conversion of n-alkanes into cell mass were determined on this basis.

Results and Discussion

Seventeen strains of yeasts were tried on n-hexadecane and kerosene. The results show that Y3/WRL, *Rhodotrula*, *Candida guilliermondii*, *Candida utilis*, *Ermothesium ashbii*, *Torulopsis magnoliae*, *Debarymyces subglobosus* and *Candida guilliermondii* were capable of utilising n-hexadecane as the sole source of carbon.

The growth of these strains on n-hexadecane was profuse (Table 1). No growth was, however, observed when kerosene was provided as the energy source. It has been reported by Scheda *et al.*[7] that yeasts could not grow on kerosene. This was attributed to the presence of certain toxic materials, branched-chain and aromatic hydrocarbons in the commercially available kerosene. Tokajiro *et al.*[8] obtained a poor growth of yeast strains on kerosene. They also reported that n-hexadecane was the most suitable carbon source. Ikunosuke *et al.*[9] observed utilisation of kerosene by yeast strains, but could not obtain appreciable cell mass. Better assimilation of n-hexadecane by yeasts has been reported by Miller *et al.*[2]

Nine strains belonging to genus Candida were found capable of utilising n-alkanes. *Torula utilis* and *Saccharomyces cerevisiae* showed positive growth but it was comparatively less than that of members of genus Candida. Candida strains have been reported as hydrocarbon utilisers by Takahashi *et al.*[5], Ikunoske *et al.*[9] Miller *et al.*[2–4] and Dostalek *et al.*[6]

Growth of Yeast on Sui Gas

Table 2 represents growth of yeasts on Sui gas. It is apparent that 5 out of 18 strains were capable

TABLE 1.—QUALITATIVE STUDY OF THE GROWTH OF YEASTS ON n-HEXADECANE.

Sl. No.	Strain No	Name	Growth	
			n-Hexadecane	Kerosene
1	0735 IFO	*Candida robusta*	++	—
2	Y3/WRL		++++	—
3	495 UAMH/6	*Rhodotrula*	++++	—
4	9058 ATCC/7	*Candida guilliermondii*	++++	—
5	39916 IMI/10	*Saccharomyces cerevisiae*	++	—
6	0621 CBS/12	*Candida utilis*	+++	—
7	1796 CBS/13	*Candida fareri*	+++	—
8	0862 NRC/15	*Torula utilis* Van	+	—
9	0193 NCYC/16	*Candida utilis*	++	—
10	0359 NCYC/17	*Candida utilis*	++++	—
11	0145 NCYC/18	*Candida guilliermondii*	+++	—
12	0944 CBS/20	*Ermothesium ashbii*	++++	—
13	0705 IFO/22	*Torulopsis magnoliae*	++++	—
14	0459 NCYC/24	*Debarymyces subglobosus*	++++	—
15	0566 IFO/30	*Candida guilliermondii*	++++	—
16	0643 IFO/31	*Candida guilliermondii*	++	—
17	0809NRC/34	*Ermothesium ashbii*	++	—

*—No growth; + turbidity persisted; ++remarkable growth; +++evident growth; ++++good growth; IFO Institute of Fermentation, Osaka, Japan; UAMH University of Alberta, Mold Herbanium and Culture Collection, Canada; ATCC Amer. Type Culture Collection, Washington, D.C. USA; IMI The Commonwealth Mycological Institute, England.; CBS Central Bureau Voor Schimmecultures, Baarn, Netherland; NRC National Research Council, Ottawa, Canada; NCYC National Collection of Yeast Cultures, Brewing Industry, Research Foundation, England; WRL West Regional Laboratories Culture Collection.

of employing gaseous hydrocarbons as a source of energy. These strains were Y3/WRL, *Candida flareri*, *Candida guilliermondii* (IFO-x30) and *Debarymyces subglobosus*. The number of Sui gas-assimilators is comparatively smaller, since yeast has a general tendency to convert higher alkanes to their cellular mass.[2] As it is evident from Table 3 that Sui gas contains 95.72% methane along with other branched chain hydrocarbons like isobutene and isopentenes. The presence of H_2S and CO_2 is also toxic for aerobes. When Sui gas was provided to the *B. subtilis*, without removing the H_2S, it inhibited the growth of the microorganisms[10]. The low solubility of Sui gas in the medium may be one of the inhibiting factors. The growth of yeasts on Sui gas was found to increase when the Medium 2 was supplemented with 0.2% peptone.

Table 2.—Screening of Natural Gas Assimilation Yeast.

Sl. No	Strain	Growth
1.	*Candida robusta*	+
2.	Y3/WRL	++++
3.	*Rhodotrula* sp.	+
4.	*Candida guilliermondii*	—
5.	*Saccharomyces cerevisiae*	—
6.	*Canadida utilis* (Henneberg) Lodder et. nan Rig.	+
7.	*Canadida flareri Torulopris candida* (Saito) Lodder	+++
8.	*Candida guilliermondii* NCYC145	++++
9.	*Torula utilis* van Thermophetes	+
10.	*Candida utilis* NCYC 193	+
11.	*Candida utilis* NCYC 359	+
12.	*Torulopris magnoliac*	+
13.	*Candida guilliermondii*	++++
14.	*Eremothesium ashbyii* Guill	+
15.	*Debarymyces subglobosus*	++++
16.	*Candida guilliermondii* IFO	+
17.	*Candida guilliermondii* van. Membrane Faciens	—
18.	*Ermothesium ashbyii* NRC 809	—

Table 3.—Composition of Sui Gas.*

CH_4	95.72%
C_3H_8	0.23%
C_2H_6	0.83%
Iso-C_4H_{10}	0.07%
n-C_4H_{10}	0.05%
n-C_5H_{12}	0.03%
Iso-C_5H_{12}	0.04%
Hexane	0.13%
Nitrogen (N_2)	3.75%
CO_2	by difference
H_2S	0.15%

Table 4.—Percentage Conversion of Hydrocarbons into Yeast Cells.

No.	Strain	Cell weight		D.M.B.		Mean* %
		A (mg)	B (mg)	A (mg)	B (mg)	
1	*C. robusta*	40.0	39.3	26.60	25.80	26.20
2	Y3/WRL	136.0	106.9	88.30	69.50	78.90
3	*Rhodotrula*	28.0	—	18.18	—	18.18
4	*C. guilliermondii*	48.0	43.8	31.16	28.40	29.78
5	*C. utilis*	28.0	—	18.18	—	18.18
6	*C. flareri*	25.4	—	16.50	—	16.50
7	*C. utilis*	29.0	44.1	18.18	28.6	22.70
8	*C. guilliermondii*	36.0	38.7	23.30	25.10	24.20
9	*Er. ashbii*	28.4	—	18.40	—	18.40
10	*Tp. magnoliae*	29.9	—	18.80	—	18.80
11	*Db. subglobosus*	—	30.1	—	19.50	19.50
12	*C. guilliermondii*	26.0	—	16.80	—	16.80

*The conversion has been calculated on the basis of hydrocarbons added, on the assumption that all the hydrocarbons are being converted into the cell mass.

Conversion of Hydrocarbons into Cell Mass

Table 4 summarises the cell yield (dry mass basis) of different strains when grown on n-hexadecane. Y3/WRL was found to give maximum cell yield (78.9%) on the basis of n-alkanes added. *Candida guilliermondii* gave the next higher cell yield of 29.78%. Other members of genus Candida gave 16–29% cell yield. Miller *et al.*[2] reported a cell yield of 82% by a hydrocarbon utiliser *Candida intermedia*. The yield was reported to have increased when a mixed culture of *Candida intermedia* and *Candida lipolytica* was used.[3,4] Continuous culture was employed for the growth of hydrocarbon utilisers by Dostalek *et al.*[6] They have reported biomass. A yield of 1.7 g/l/hr with yield coefficient 0.92 was reported by these workers.

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