Mycological Fat Production in India. II. Effect of Hydrogen-Ion Concentration on Fat Synthesis

By Krishna Nand and B. S. Mehrotra

Abstract

The influence of pH on the dry weight of mycelium, fat content, and sugar utilization by *Mucor circinelloides* van Tiegh., *M. recurvus* Butler, *M. peacockensis* Meh. and Nand, *Phycomyces blakesleeanus* Burgeff, *Aspergillus allahabadii* Meh. and Agni., *A. indicus* Meh. and Agni., and *Penicillium oxalicum* Currey et Thom was studied. All these species grew on a wide range of pH (2.0—13.0), but the good yield of fat and felt was observed at pH range 4.0—9.0. Two of the species namely *Mucor recurvus* and *Phycomyces blakesleeanus* gave good yield of fat at pH 6.5 while other five species yielded maximum at pH 8.5. However, pH 6.5 was selected for further studies on fat production, as both the economic and fat coefficients in all the species were found to be highest at this pH.

Introduction

Like growth, fat formation is also controlled to some extent by the pH of the nutrient medium. Higher concentrations of hydrogen or hydroxyl ions inhibit the growth and also hamper fat formation. The optimum pH for fat synthesis varies greatly with the organisms. Kleinzeller (1949) has reported that highest fat formation occurs at pH 5.5 to 6.0, and the conversion coefficient also decreases with the increasing pH. Prill *et al.* (1935) found higher fat formation at higher range of pH. Working with *Mucor mucedo*, Blinc and Bojec (1942) found that at pH 7.0 with maltose, dextrose, wort or molasses as carbon sources, fat synthesis was high and was very similar to seed fat. Cioffi and Varetto (1951) working on *Penicillium javanicum* found pH values, 4.5—5.5 as best for fat synthesis in surface cultures. Steinberg and Ordal (1954) found that fat synthesis by *Rhodotorula gracilis* varied linearly with pH 3.0—6.5.

In the present study, the fat-forming fungi *Aspergillus allahabadii* Meh. and Agni., *A. indicus* Meh. and Agni., *Mucor circinelloides* van Tiegh., *M. peacockensis* Meh. and Nand., *M. recurvus* Butler, *Penicillium oxalicum* Currey et Thom and *Phycomyces blakesleeanus* Burgeff, were taken and an attempt was made to study the effects of different pH values on fat and felt formation.

Materials and Methods

The organisms were maintained on Czapek's solution agar and oatmeal agar media. The culture media had the following composition (g./1000 ml): (a) NH_4NO_3, 3.0; KCl, 2.0; $MgCl_2$. $6H_4O$, 0.04; Na_2SO_4, 0.1; NaH_2PO_4. H_2O, 0.2; $ZnSO_4$. $7H_2O$, 4.5 mg; $FeSO_4$. $7H_2O$, 5.0 mg; $CuSO_4$. $5H_2O$, 0.08 mg; sucrose, 25; and distilled water, 1000 ml. (b) KH_2PO_4, 0.5; $MgSO_4$. $7H_2O$, 0.25; asparagine, 2.0; thiamin chloride, 0.5 mg; glucose, 25; and distilled water, 1000 ml. Thirty ml. portions of each medium were poured into 150 ml Erlenmeyer flasks. The hydrogenion concentrations of the media varied from pH 2.8—13.0 and different pH values were adjusted by N 10 NaOH or N 10 HCl after the sterilization of the fiasks at 15 lbs pressure for 15 minutes. The pH values of the nutrient solution were determined by using Mercks, Reagenzien pH paper. The species of *Aspergillus* and *Penicillium* were grown on medium (1) while other species were grown on medium (b). Replicates in triplicate were taken in each case.

The method of inoculation, incubation, harvesting, drying, grinding, fat extraction, and sugar estimation was the same as in the first paper of this series (Mehrotra and Nand, 1970). Economic and fat coefficients were calculated according to the formulae proposed by Foster (1949) and Rippel (1940) respectively. The results were statistically analyzed by the method recommended by Paterson (1939).

The general mean of the experiment $\pm$ C. D. at 1% level has been taken as moderate. This served as a measure for classifying the data of different experiments into good and poor. The dry weights, sugar utilization, and fat percentages higher or lower than the moderate have been designated as good or poor respectively.

Results and Discussion

The design of the experiments was factorial. The arithmetic mean of three replicate yields of dry mycelium, single reading of fat yields (mg./100 mg dry felt) and sugar utilization (mg/per flask) are listed in Tables I—VII and graphically represented in Figures 1—7. A statistical test showed that for all species which were taken in this experiment, growth at pH 6.0—7.0 was best while maximum yields of fat and high sugar utilization was observed at pH 6.0—8.5.

All the treatments are statistically significant at 5 and 1 percent levels.

The optimum pH range for good growth and fat formation varies with species of fungi. pH between 6.5—8.5 was good for fat synthesis by *Mucor peacockensis* and *Phycomyces blakesleeanus*. Similarly pH values varying from 6.0—9.0, 8.5—9.0 and 6.0—8.0 seemed favourable for fat synthesis by *Aspergillus indicus*, *A. allahabadii* and *Mucor*

recurvus respectively. For *Penicillium oxalicum*, *Mucor circinelloides*, however, a more wide range of pH, i. e., 4.5—9.0 was suitable for fat formation. On the other hand, the optimum pH for good growth varies from 5.0—7.0 for *Mucor circinelloides* and *Aspergillus indicus*, 4.5—7.0 for *Phycomyces blakesleeanus* and *Penicillium oxalicum*, 4.0—7.0 for *Mucor peacockensis*, 5.0—8.0 for *Mucor recurvus* and 6.0—8.5 for *Aspergillus allahabadii*. It has been found by Steinberg and Ordal (1954) that fat formation varied linearly with pH (3.0—8.5) Prill *et al.*, (1935) and Ward, Lockwod *et al.*, (1935) have also reported that neutral or slightly alkaline pH were most favourable for fat synthesis by *Aspergillus fischeri* and *Penicillium javanicum*. Litvinova and Raevskaya (1952) found pH 3.0—8.0 good for fat synthesis by some species of *Fusarium*.

The present species of fungi may be categorized into two groups on the basis of their pH requirement at which they show maximum fat formation.

(a) Species requiring nearly neutral pH

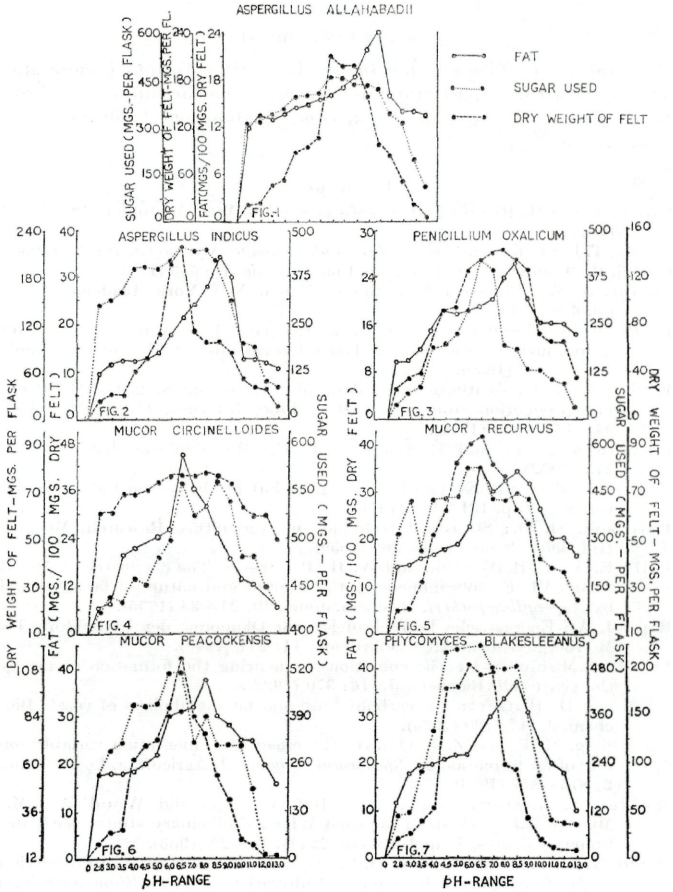
In this category can be placed *Mucor recurvus* and *Phycomyces blakesleeanus* in which highest yields of fat and felt and maximum sugar utilization were observed at pH 6.0 and 6.5 respectively. Kleinzeller (1948) also found in the case of *Torulopsis lipofera*, maximum felt and fat formation at pH 5.5—6.0. So was the case with *Aspergillus ustus* and *Penicillium notatum* which produced maximum felt and fat at pH 6.4 and 5.8 respectively (Wix and Woodbine, 1959). Brock (1956) also reported in *Hansenula anomala*, a pH of 6.5 to be most favourable for cell material and lipid synthesis.

(b) Species requiring nearly alkaline pH

In this category can be placed five species, namely *Mucor circinelloides*, *M. peacockensis*, *Aspergillus allahabadii*, *A. indicus* and *Penicillium oxalicum*. In these, maximum growth and high sugar utilization was observed at pH 6.5, while maximum fat synthesis was reached at pH 8.5. These species had an initial stage of growth with little fat formation, followed by a stage of high fat accumulation after the maximum felt weight has been reached. It is evident from the Figs. 1, 2, 3, 4 and 6 that there is an inverse relationship between fat synthesis, growth and sugar utilization, fat percentage being highest and sugar utilization and growth lowest at pH 8.5. On the other hand, at pH 6.5, growth and sugar utilization were the highest and fat percentage was the lowest. Similar findings were reported by Blinc and Bojec that pH 7.0 was the best for fat production by *Mucor mucedo*. Ikeda (1950) has also concluded that pH towards acidic side was necessary for growth and neutral to slightly alkaline was favourable for fat synthesis by *Aspergillus candidus*.

At lower pH, fat synthesis was considerably inhibited (Tables I, III, IV, VI and VII). Similar observations have been made by a

number of workers (Foster, 1949; Smedley-Maclean and Hoffert, 1923). Walker, Wix and Woodbine (1959) have stated that fat production does not occur in cultures where the pH falls, due to the conversion of sugars to organic acids. The conversion of carbohydrate to lipid or lipid to carbohydrates was observed by Smedley-Maclean (1922) in *Saccharomyces* species, who stated that possibly at



low pH, lipid synthesis is inhibited and the assimilated glucose converted to other carbohydrates; while at higher pH lipid synthesis is favoured and more of the glucose is used for fat synthesis reducing the conversion of glucose to other carbohydrates.

Although for maximum fat formation, two different pH values for the organisms were observed, yet the economic and fat coefficients were found to be highest at pH 6.5 for all the species as the growth was good at this pH.

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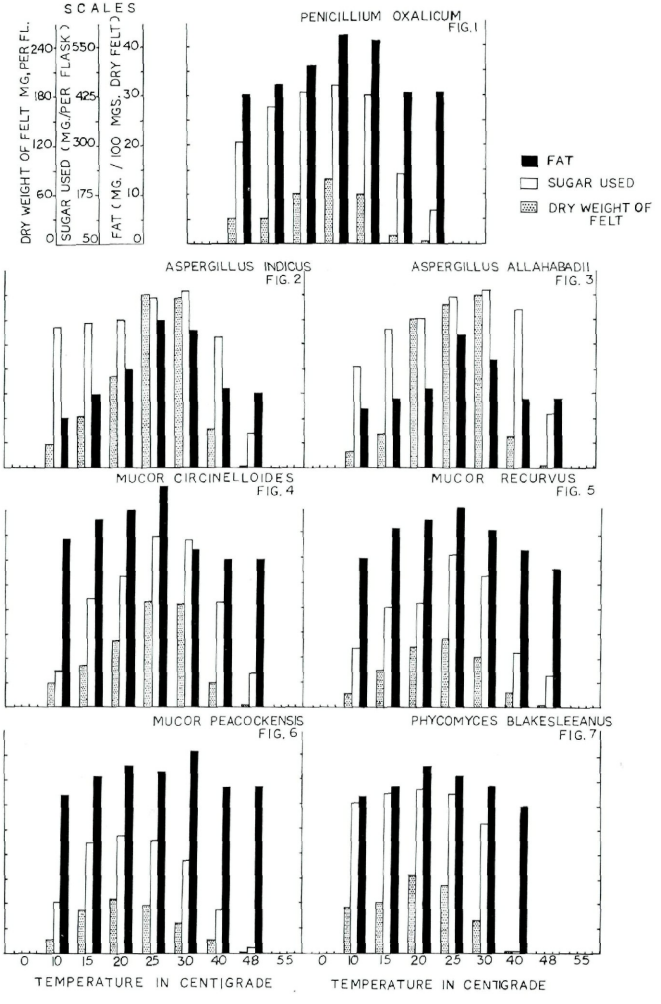
HISTOGRAM SHOWING DRY WEIGHT OF FELT AND SUGAR UTILIZATION AT DIFFERENT TEMPERATURES



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Platte VI.

The effect of pH on the growth, sugar utilization and fat production of *Penicillium oxalicum*, *Aspergillus allahabadii*, *A. indicus*, *Mucor circinelloides*, *M. recurvus*, *M. peacockensis* and *Phycomyces blakesleeanus*.

Table I. Influence of pH of the medium on fat synthesis, felt formation and sugar utilization by *Aspergillus allahabadii* Meh. and Agni

(Vol. of culture medium, 30 ml; incubation period, 16 days; incubation temp. 25° C)

Initial pH	Final pH	Sugar utilized (mg/per flask)	Dry wt of felt (mg/per flask)	Fat (mg/100 mg dry felt)	Economic coeffi-cient	Fat coeffi-cient
2.8	2.2	310.0	21.2	12.248	6.838	1.410
3.0	2.5	323.0	24.2	13.322	7.492	1.675
3.5	2.8	342.0	46.2	13.320	13.508	3.540
4.0	3.0	389.0	52.4	14.210	13.470	3.554
4.5	3.5	400.2	90.8	14.620	22.682	4.261
5.0	4.0	400.8	98.6	14.820	24.501	4.459
6.0	5.5	412.4	108.6	15.320	26.336	5.084
6.5	5.8	468.2	210.4	16.280	44.976	6.236
7.0	6.5	460.4	200.8	17.240	43.307	4.216
8.0	7.0	440.7	183.4	18.400	48.307	4.216
8.5	7.2	438.2	160.2	22.000	36.558	3.906
9.0	8.0	421.2	100.2	24.000	23.789	3.020
10.0	8.2	350.2	85.2	16.000	24.557	2.246
11.0	8.5	321.2	48.2	15.240	15.006	2.025
12.0	8.5	202.2	23.2	15.200	11.473	2.000
13.0	8.5	116.4	6.4	14.000	5.498	1.403
C. D.	5%	17.232	13.754	2.525		
	1%	20.286	15.054	3.033		

Table II. Influence of pH of the medium on fat synthesis, felt formation and sugar utilization by *Aspergillus indicus* Meh. & Agni.
(Vol. of culture medium, 30 ml; incubation period, 16 days; incubation temp. 25° C)

Initial pH	Final pH	Sugar utilized (mg/per flask)	Dry wt of felt (mg/per flask)	Fat (mg/100 mg dry felt)	Economic coeffi-cient	Fat coeffi-cient
2.8	2.2	310.0	24.4	10.200	7.876	0.802
3.0	2.8	322.0	31.8	12.460	9.875	1.230
3.5	2.8	338.0	32.0	12.540	9.175	4.012
4.0	3.5	400.6	60.8	12.830	15.107	1.947
4.5	3.8	412.0	80.2	12.920	19.466	2.515
5.0	4.0	410.0	108.6	14.620	26.487	3.872
6.0	4.5	420.0	198.2	18.420	47.190	8.692
6.5	4.8	460.0	220.4	22.320	47.913	10.694
7.0	5.5	450.0	116.4	24.720	24.250	6.339
8.0	6.5	458.0	96.6	28.400	21.528	6.610
8.5	7.0	450.2	86.4	34.840	30.437	7.976
9.0	7.5	315.2	52.8	30.840	27.411	8.453
10.0	7.8	210.6	42.6	13.860	24.071	3.474
11.0	8.0	208.4	40.6	13.000	24.414	2.657
12.0	8.5	110.2		12.000	38.842	4.421
13.0	8.5	90.6	20.8	11.240	22.957	2.599
C. D.	5%	22.462	10.482	2.984		
	1%	27.946	15.948	3.368		

Table III. Influence of pH of the medium on fat synthesis, felt formation and sugar utilization by *Penicillium oxalicum* Currey et Thom.
(Vol. of culture medium, 30 ml; incubation period, 16 days; incubation temperature, 25° C)

Initial pH	Final pH	Sugar utilized (mg/per flask)	Dry wt of felt (mg/per flask)	Fat (mg/100 mg dry felt)	Economic coeffi-cient	Fat coeffi-cient
2.8	3.0	112.0	12.6	10.210	11.250	1.147
3.0	3.4	114.0	22.8	10.260	20.000	1.624
3.5	5.8	118.0	29.2	12.240	24.745	3.028
4.0	5.5	248.0	62.4	14.600	25.161	3.673
4.5	5.8	292.0	64.6	18.200	22.123	4.086
5.0	8.2	301.0	72.0	18.320	24.252	4.370
6.0	8.2	391.0	92.4	18.480	23.631	4.350
6.5	8.2	421.0	134.2	19.320	31.878	6.134
7.0	8.2	330.0	126.2	20.240	28.681	5.759
8.0	8.5	445.0	62.0	24.220	13.056	3.317
8.5	8.6	422.0	60.0	26.220	14.218	3.727
9.0	8.8	399.0	42.0	20.010	10.551	2.106
10.0	8.8	224.0	42.0	16.240	18.750	3.044
11.0	8.8	212.0	36.2	16.200	17.075	2.766
12.0	9.0	210.0	30.2	16.100	14.380	1.610
13.0	9.0	119.0	10.2	14.420	8.571	1.235
C. D.	5%	3.028	1.915	0.529		
	1%	3.148	2.580	0.703		

Table IV. Influence of pH of the medium on fat synthesis, felt formation and sugar utilization by *Mucor circinelloides* van Tieghem

(Vol. of culture medium, 25 ml.; incubation period, 16 days; incubation temp. 25° C)

Initial pH	Final pH	Sugar utilized (mg/per flask)	Dry wt of felt (mg/per flask)	Fat (mg/100 mg dry felt)	Economic coeffi-cient	Fat coeffi-cient
2.8	2.0	424.0	20.0	20.123	4.761	1.420
3.0	2.5	444.0	24.6	30.246	5.540	1.675
3.5	3.0	434.0	44.6	34.456	10.276	3.540
4.0	3.5	462.0	46.2	35.546	10.000	3.554
4.5	3.8	452.0	52.4	36.764	13.805	4.261
5.0	4.0	482.0	55.8	38.524	11.567	4.459
6.0	5.5	498.0	62.8	40.321	12.610	5.084
6.5	6.0	560.0	86.4	40.420	11.214	6.236
7.0	6.6	522.0	71.2	40.628	13.639	6.086
8.0	6.6	536.0	54.8	41.242	10.233	4.216
8.5	6.8	562.0	54.6	40.202	9.715	3.906
9.0	7.0	542.0	42.6	38.424	7.859	3.020
10.0	8.0	488.0	34.0	32.242	6.906	2.246
11.0	8.2	476.0	32.0	30.124	6.723	2.025
12.0	8.8	450.0	30.0	30.000	6.666	2.000
13.0	9.0	430.0	21.2	28.464	4.930	1.403
C. D.	5%	10.433	4.292	2.754		
	1%	14.049	5.780	3.709		

Table V. Influence of pH of the medium on fat synthesis, felt formation and sugar utilization by *Mucor recurvus* Butler

(Vol. of culture medium, 30 ml.; incubation period, 16 days; incubation temp. 25° C)

Initial pH	Final pH	Sugar utilized (mg/per flask)	Dry wt of felt (mg/per flask)	Fat (mg/100 mg dty felt)	Economic coeffi-cient	Fat coeffi-cient
2.8	2.5	321.0	20.0	14.246	6.230	0.887
3.0	3.5	426.0	21.0	14.538	4.928	0.716
3.5	4.0	262.0	32.0	16.234	12.213	1.982
4.0	4.5	428.0	52.8	17.242	12.336	2.126
4.5	4.8	429.0	62.0	18.268	14.452	2.410
5.0	5.5	429.0	82.0	19.200	19.112	3.650
6.0	6.2	528.0	90.4	35.620	17.410	3.977
6.5	6.2	528.0	92.4	34.646	15.569	3.837
7.0	6.5	430.0	81.0	30.240	18.837	5.696
8.0	6.5	432.0	76.2	32.400	17.639	5.714
8.5	6.5	446.0	62.4	33.620	13.400	4.703
9.0	8.0	430.0	56.0	31.424	13.400	4.092
10.0	8.5	312.0	26.2	20.424	8.397	2.186
11.0	9.0	212.0	24.0	20.822	11.320	2.357
12.0	9.2	198.0	19.8	20.104	10.000	2.014
13.0	9.2	111.0	10.2	16.234	9.180	1.491
C. D.	5%	5.446	4.547	1.351		
	1%	7.046	6.124	1.820		

Table VI. Influence of the pH of the medium on fat synthesis, felt formation and sugar utilization by *Mucor peacockensis* Meh. and Nand.
(Vol. of culture medium, 30 ml.; incubation period, 16 days; incubation temp. 25° C)

Initial pH	Final pH	Sugar utilized (mg/per flask)	Dry wt of felt (mg/per flask)	Fat (mg/100 mg dry felt)	Economic coeffi-cient	Fat coeffi-cient
2.8	2.2	232.0	20.2	18.624	8.706	1.620
3.0	2.2	300.0	25.2	18.800	8.400	1.578
3.5	3.2	320.0	30.6	18.482	9.500	1.767
4.0	3.5	424.0	62.4	19.324	14.710	4.851
4.5	4.1	416.0	68.0	20.412	16.346	3.363
5.0	4.5	428.0	76.2	22.346	17.803	3.967
6.0	5.5	512.0	96.8	30.230	18.710	5.734
6.5	6.1	326.0	112.6	31.544	21.992	6.939
7.0	6.6	326.0	84.6	32.426	22.573	8.414
8.0	7.7	394.0	56.2	38.262	14.263	5.714
8.5	7.7	312.0	54.2	30.200	17.371	6.864
9.0	8.0	310.0	42.6	28.126	13.741	3.800
10.0	8.2	308.0	22.4	25.000	7.272	1.816
11.0	8.5	195.0	20.0	25.000	10.420	1.333
12.0	9.5	10.0	14.2	20.242	14.200	2.874
13.0	9.5	10.0	12.0	16.420	12.000	1.970
	5%	3.310	8.261	5.400		
C. D.						
	1%	4.457	11.126	6.301		

Table VII. Influence of pH of the medium on fat synthesis, felt formation and sugar utilization by *Phycomyces blakesleeanus* Burgeff
(Vol. of culture medium, 30 ml; incubation period, 16 days; incubation temp. 25° C)

Initial pH	Final pH	Sugar utilized (mg/per flask)	Dry wt of felt (mg/per flask)	Fat (mg/100 mg dry felt)	Economic coeffi-cient	Fat coeffi-cient
2.8	2.0	112.0	24.2	12.624	21.607	2.727
3.0	2.5	114.0	28.6	18.240	25.087	4.567
3.5	3.0	224.0	42.6	20.320	19.017	3.864
4.0	3.5	328.0	61.2	20.420	18.658	3.809
4.5	4.0	526.0	181.2	21.420	34.448	7.759
5.0	4.5	528.0	181.2	22.410	34.316	7.691
6.0	5.1	456.0	194.6	22.520	42.675	9.610
6.5	5.5	540.0	198.2	30.628	41.531	11.676
7.0	6.5	480.0	197.6	30.242	41.166	12.449
8.0	6.8	480.0	100.6	34.464	20.958	7.223
8.5	6.8	482.0	94.6	37.464	19.626	8.397
9.0	7.0	370.0	42.2	30.212	11.351	3.445
10.0	8.0	210.0	21.2	24.624	12.277	2.486
11.0	8.2	101.0	12.4	20.211	12.277	1.476
12.0	8.5	100.0	10.0	18.210	10.000	1.821
13.0	8.5	98.0	10.0	10.420	10.200	1.063
	5%	6.211	9.795	7.477		
C. D.						
	1%	8.365	13.191	Not significant		