PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Quantitative Examination by the Carbon Balance Sheet Method of the Types of Products Formed from Glucose by Species of Fusarium

John Howard Birkinshaw, John Henry Victor Charles, Harold Raistrick and John Alexander Robertson Stoyle

Phil. Trans. R. Soc. Lond. B 1931 **220**, 93-98
doi: 10.1098/rstb.1931.0016

Studies in the Biochemistry of Micro-organisms.

PART V.—*Quantitative examination by the carbon balance sheet method of the types of products formed from glucose by species of Fusarium.*

By JOHN HOWARD BIRKINSHAW, JOHN HENRY VICTOR CHARLES, HAROLD RAISTRICK, *and* JOHN ALEXANDER ROBERTSON STOYLE.

In view of the encouraging results obtained from the preparation of carbon balance sheets for a number of species of *Aspergillus*, as reported in Part III, the work was extended to other groups and, by way of contrast, carbon balance sheets were prepared for a considerable number of species of *Fusarium*. It is evident from WOLLENWEBER'S "Monograph on the *Fusaria*" (see 'Phytopathology' (1913), Vol. 3, p. 24) that the classification of the *Fusaria* on morphological grounds is very difficult. It was hoped that, as a result of the preparation of carbon balance sheets, some biochemical classification on the lines of the *Aspergillus* group might be possible, and in order to ensure that the cultures used were authentic, almost the whole of those chosen for investigation were WOLLENWEBER'S own cultures purchased from the Centraalbureau voor Schimmelcultures at Baarn. The methods of investigation adopted were those described in Part II and applied to the *Aspergillus* group in Part III.

The following is the history of the species of *Fusarium* used in this work :—

*(1) *F. viride* (LECHM.) WR., Catalogue No. Ag. 81. Purchased from Centraalbureau voor Schimmelcultures at Baarn.
*(2) *F. solani* MART. var. *minus* WR., Catalogue No. Ag. 83. Purchased from Baarn.
(3) *F. lini* BOLLEY, Catalogue No. Ag. 60. Purchased from British National Collection of Type Cultures, No. 1082.
*(4) *F. Martii* APP. et WR., Catalogue No. Ag. 77. Purchased from Baarn.
(5) *F.* species, Catalogue No. Ag. 80. Isolated at Ardeer from infected potato haulm.
(6) *F. dianthi* PRILL. et DEL., Catalogue No. Ag. 59. Purchased from British National Collection of Type Cultures No. 1136.
*(7) *F. uncinatum* WR., Catalogue No. Ag. 75. Purchased from Baarn.
*(8) *F. trichothecioides* WR., Catalogue No. Ag. 73. Purchased from Baarn.
*(9) *F. tubercularioides* (CORDA) SACC., Catalogue No. Ag. 72. Purchased from Baarn.
*(10) *F. cœruleum* (LIB.) SACC., Catalogue No. Ag. 65. Purchased from Baarn.
*(11) *F. salicis* FUCK., Catalogue No. Ag. 69. Purchased from Baarn.
*(12) *F. javanicum* KOORDERS, Catalogue No. Ag. 78. Purchased from Baarn.

*(13) *F. falcatum* APP. et WR., Catalogue No. Ag. 82. Purchased from Baarn.
*(14) *F. avenaceum* (FR.) SACC., Catalogue No. Ag. 76. Purchased from Baarn.
(15) *F. sporotrichoides* SHERB., Catalogue No. Ag. 58. Purchased from British National Collection of Type Cultures, No. 1296.
*(16) *F. sambucinum* FUCK., Catalogue No. Ag. 70. Purchased from Baarn.
(17) *F. vasinfectum* ATK., Catalogue No. Ag. 71. Purchased from Baarn.
*(18) *F. rhizophilum* CORDA, Catalogue No. Ag. 74. Purchased from Baarn.
*(19) *F. metachroum* APP. et WR., Catalogue No. Ag. 68. Purchased from Baarn.
(20) *F.* species, Catalogue No. Ag. 61. Isolated at Ardeer from a rotting potato.
*(21) *F. orthoceras* APP. et WR., Catalogue No. Ag. 66. Purchased from Baarn.
(22) *F. scirpi* LAMB et FAUTR., Catalogue No. Ag. 79. Purchased from Baarn.
(23) *F. oxysporum* SCHLECHT, Catalogue No. Ag. 67. Purchased from Baarn.

Fifteen of the cultures in the above list which are marked with an asterisk were WOLLENWEBER'S cultures purchased from Baarn.

The carbon balance sheets prepared for these species are given in Tables I and II.

Discussion of results obtained.

None of the species grew very well on the synthetic CZAPEK-DOX metabolism solution used, as is evidenced by the relatively small weights of mycelium obtained, compared with the corresponding figures for species of *Aspergillus*. It is significant, however, that in spite of the relatively small growth of mycelium, the amount of glucose metabolized was very considerable.

The only metabolic product produced in quantity by any of the *Fusaria* is in the group known as "volatile neutral compounds," and in one or two instances where investigation as to its nature took place, it was shown to be ethyl alcohol. The carbon balance sheets show, indeed, that, biochemically speaking, the *Fusaria* function in a very similar manner to the *Saccharomyces*, and in many cases the yields of alcohol formed from glucose by species of *Fusarium* compare favourably with the corresponding yields given by the best species of *Saccharomyces*. Thus, with *F. avenaceum* (Ag. 76), of 4·638 gm. of carbon as glucose metabolized, 2·453 gm. appear as "volatile neutral compounds" representing a yield of 52·9 per cent. This yield is even larger if, in calculating it, allowance is made for the carbon utilised by the fungus in building up its mycelium. Calculated on these lines, the yield is 55·1 per cent. Almost theoretical values could, no doubt, be obtained if ready-grown mycelia were used. The theoretical yield is 66·7 per cent., and the maximum yield obtained with species of *Saccharomyces* is about 60 per cent.

The property of the *Fusaria* of producing large quantities of alcohol from glucose might be turned to account technically in the production of alcohol from waste vegetable matter. Many species of *Fusarium* attack vegetable matter in the raw state without

TABLE I.—Carbon balance sheets for various species of *Fusarium*.

Species of ***Fusarium*** :	*F. viride.*	*F. solani*, var. *minus*.	*F. lini.*	*F. Martii.*	*F.* species (from potato).	*F. dianthi.*	*F. uncinatum.*	*F. trichothecioides.*	*F. tuberculari-oides.*	*F. cœruleum.*	*F. salicis.*	*F. javanicum.*
Catalogue number :	Ag. 81	Ag. 83	Ag. 60	Ag. 77	Ag. 80	Ag. 59	Ag. 75	Ag. 73	Ag. 72	Ag. 65	Ag. 69	Ag. 78
Experiment number :	A 25	A 27	A 29	A 21	A 24	A 3	A 19	A 18	A 28	A 9	A 13	A 22
Incubation period in days :	43	51	56	51	72	34	34	41	41	32	52	36
Carbon Balance Sheet.												
Carbon in solution (start) ... ... gm.	4·901	4·901	4·901	4·901	4·901	5·018	5·018	5·018	4·901	5·018	5·018	4·901
Carbon in H_2SO_4 ... ... ,,	0·003	0·005	0·009	0·010	0·002	0·020	0·019	0·011	0·017	0·016	0·015	0·019
,, in CO_2 ... ... ,,	1·053	1·204	1·652	1·361	1·162	1·654	1·487	1·279	1·532	1·380	1·178	1·613
,, in mycelium... ... ... ,,	0·382	0·430	0·450	0·267	0·385	0·373	0·216	0·137	0·195	0·392	0·107	0·285
,, in solution (end) ... ... ,,	3·439	3·175	2·642	3·161	3·317	2·794	3·143	3·461	3·077	3·093	3·577	2·802
,, accounted for ... ... ,,	4·877	4·814	4·753	4·799	4·866	4·841	4·865	4·888	4·821	4·881	4·877	4·719
,, accounted for ... per cent.	99·5	98·2	97·0	97·9	99·3	96·5	97·0	97·4	98·4	97·3	97·2	96·3
Analysis of Solution.												
Carbon in residual glucose ... gm.	2·410	1·948	0·945	1·428	2·564	0·712	0·380	0·744	0·086	0·641	1·035	0·143
,, in CO_2 in solution ... ... ,,	0·005	0·005	0·000	0·004	0·008	0·011	0·017	0·021	0·006	0·012	0·018	0·017
,, in volatile acids ... ... ,,	0·198	0·212	0·175	0·130	0·109	0·088	0·010	0·053	0·070	0·018	0·097	0·034
,, in non-volatile acids ... ,,	0·054	0·067	0·078	0·076	0·057	0·084	0·251	0·247	0·241	0·237	0·187	0·169
,, in volatile neutral compounds ,,	0·368	0·488	1·006	1·183	0·109	1·580	2·228	2·120	2·362	1·870	2·126	2·246
,, in synthetic compounds ... ,,	0·074	0·144	0·124	0·132	0·059	0·151	0·123	0·082	0·183	0·132	0·043	0·202
Total carbon accounted for ... ,,	3·109	2·864	2·328	2·953	2·906	2·626	3·009	3·267	2·948	2·910	3·506	2·811
,, ,, in solution ... ... ,,	3·439	3·175	2·642	3·161	3·317	2·794	3·143	3·461	3·077	3·093	3·577	2·802
Carbon unaccounted for (by difference),,	0·330	0·311	0·314	0·208	0·411	0·168	0·134	0·194	0·129	0·183	0·071	Surplus of 0·009
Residual Glucose.												
Glucose (by polarimeter) ... per cent.	1·308	1·040	0·504	0·726	1·383	0·428	0·199	0·320	0·018	0·378	0·493	0·139
,, (Shaffer-Hartmann) ,,	1·205	0·974	0·473	0·714	1·282	0·356	0·190	0·372	0·043	0·320	0·517	0·072
,, (Wood-Ost) ... ,,	1·260	1·052	0·496	0·744	1·286	0·369	—	0·373	—	0·311	0·508	—
,, (by alkaline iodine) ,,	1·257	1·035	0·522	0·769	1·361	0·384	0·315	0·539	0·266	0·454	0·717	0·261
Acids.												
Titration (N/1 acid) ... ... c.c.	4·9	4·8	4·1	4·7	1·6	1·0	7·1	6·5	5·4	6·1	5·2	0·9
Volatile acids (N/1 acid) ... ... ,,	9·54	9·07	8·20	7·00	5·69	0·33	1·03	1·86	2·92	0·26	3·30	1·32
Barium salts (weight) ... ... gm.	1·045	1·089	0·925	0·698	0·552	0·027	0·027	0·204	0·307	0·053	0·409	0·121
Calcium salts (weight) ... ... ,,	—	0·329	0·289	0·537	0·193	0·353	1·162	1·008	0·961	1·131	0·783	0·831
Volume of oxygen absorbed ... c.c.	1362	1557	1814	1274	1838	1678	630	463	564	705	345	784
Respiration coefficient	1·45	1·45	1·70	2·00	1·19	1·85	4·46	5·24	5·10	3·69	6·45	3·88
Mycelium (weight) ... ... gm.	0·732	0·849	0·918	0·505	0·709	0·718	0·407	0·258	0·366	0·762	0·199	0·514
,, (carbon) ... per cent.	52·2	50·6	49·0	53·0	54·3	51·9	53·0	53·1	53·2	51·4	53·9	55·5

TABLE II.—Carbon balance sheets for various species of *Fusarium*.

Species of *Fusarium*:	*F. falcatum.*	*F. avenaceum.*	*F. sporotrichoides.*	*F. sambucinum.*	*F. vasinfectum.*	*F. rhizophilum.*	*F. metachroum.*	*F.* species.	*F. orthoceras.*	*F. scirpi.*	*F. oxysporum.*
Catalogue number:	Ag. 82	Ag. 76	Ag. 58	Ag. 70	Ag. 71	Ag. 74	Ag. 68	Ag. 61	Ag. 66	Ag. 79	Ag. 67
Experiment number:	A 26	A 20	A 4	A 14	A 15	A 17	A 12	A 6	A 10	A 23	A 11
Incubation period in days:	56	39	67	45	58	70	66	71	40	33	50
Carbon Balance Sheet.											
Carbon in solution (start) ... ... gm.	4·901	4·901	5·018	5·018	5·018	5·018	5·018	5·018	5·018	4·901	5·018
Carbon in H_2SO_4 ... ... ,,	0·021	0·018	0·028	0·034	0·015	0·015	0·026	0·033	0·024	0·023	0·031
,, in CO_2 ... ... ,,	1·550	1·536	1·485	1·617	1·486	1·104	1·240	1·273	1·831	1·826	1·965
,, in mycelium... ... ... ,,	0·281	0·187	0·386	0·208	0·276	0·211	0·199	0·145	0·207	0·453	0·269
,, in solution (end) ... ... ,,	2·989	3·085	2·980	2·974	3·118	3·634	3·463	3·490	2·851	2·452	2·589
,, accounted for ... ... ,,	4·841	4·826	4·879	4·833	4·895	4·964	4·928	4·941	4·913	4·754	4·854
,, accounted for ... per cent.	98·8	98·5	97·2	96·3	97·6	99·0	98·2	98·5	97·9	97·0	96·7
Analysis of Solution.											
Carbon in residual glucose ... gm.	0·056	0·263	1·158	0·129	0·892	1·838	1·530	1·575	0·077	0·025	0·004
,, in CO_2 in solution ... ... ,,	0·010	0·019	0·003	0·013	0·015	0·008	0·005	0·009	0·018	0·021	0·031
,, in volatile acids ... ... ,,	0·035	0·027	0·033	0·032	0·029	0·018	0·009	0·018	0·025	0·033	0·021
,, in non-volatile acids ... ,,	0·065	0·127	0·140	0·125	0·112	0·070	0·078	0·084	0·107	0·102	0·117
,, in volatile neutral compounds ,,	2·362	2·453	1·389	2·464	1·846	1·528	1·684	1·612	2·332	2·078	2·165
,, in synthetic compounds ... ,,	0·191	0·098	0·092	0·104	0·079	0·129	0·063	0·080	0·095	0·130	0·058
Total carbon accounted for ... ,,	2·719	2·987	2·815	2·867	2·973	3·591	3·369	3·378	2·654	2·389	2·396
,, ,, in solution ... ... ,,	2·989	3·085	2·980	2·974	3·118	3·634	3·463	3·490	2·851	2·452	2·589
Carbon unaccounted for (by difference),,	0·270	0·098	0·165	0·107	0·145	0·043	0·094	0·112	0·197	0·063	0·193
Residual Glucose.											
Glucose (by polarimeter) ... per cent.	0·037	0·219	0·748	0·029	0·460	0·973	0·767	0·767	0·099	0·106	0·018
,, (SHAFFER-HARTMANN) ,,	0·028	0·131	0·579	0·064	0·446	0·919	0·765	0·787	0·039	0·013	0·002
,, (WOOD-OST) ... ,,	—	—	0·598	—	0·444	0·950	0·778	0·803	—	—	—
,, (by alkaline iodine) ,,	0·112	0·215	0·740	—	0·550	0·966	0·829	0·836	—	0·093	—
Acids.											
Titration (N/1 acid) ... ... c.c.	4·1	3·2	2·3	1·9	1·5	1·0	0·9	0·8	0·3	Decrease of 0·7	Decrease of 1·3
Volatile acids (N/1 acid) ... ... ,,	2·15	1·47	0·95	1·52	1·58	1·33	0·73	0·66	0·87	2·04	1·96
Barium salts (weight) ... ... gm.	0·186	0·135	0·093	0·133	0·125	0·074	0·059	0·053	0·051	0·177	0·180
Calcium salts (weight) ... ... ,,	0·675	0·751	0·655	0·689	0·656	0·486	0·453	0·361	0·526	0·515	0·684
Volume of oxygen absorbed ... c.c.	623	532	1465	641	1018	574	699	923	1047	1090	1339
Respiration coefficient	4·67	5·46	1·90	4·74	2·76	3·61	3·32	2·59	3·30	3·17	2·78
Mycelium (weight) ... ... gm.	0·530	0·361	0·677	0·375	0·456	0·346	0·358	0·278	0·384	0·877	0·518
,, (carbon) ... ... per cent.	53·0	51·9	57·0	55·3	60·5	61·2	55·7	52·1	53·8	51·7	51·9

preliminary hydrolysis by some other agent, and it is conceivable that, since they also ferment pentoses with the production of alcohol, some species of *Fusarium* might prove to be a suitable organism for solving the problem of obtaining alcohol from waste vegetable material.

Perusal of the balance sheets shows that, unlike the *Aspergilli*, no classification of the *Fusaria* is possible on the lines of production or non-production of alcohol (" volatile neutral compounds ") since of the twenty-three species examined all give appreciable quantities of alcohol, and almost all give very good yields indeed. However, though no satisfactory classification of the *Fusaria* appears to be possible from the carbon balance sheets presented, it is possible to separate a few species which have rather unusual characteristics. For this reason the different species examined are arranged in the two tables in the following manner :—The species are arranged in order of decreasing acid production, the first members of Table I being those which give rise to the largest amounts of " volatile acids," and immediately succeeding them come the species which give rise to " non-volatile acids," so that at the one end of the table are placed those species giving the largest amount of volatile acids, while at the other end are those species which give very little acidity either of a volatile or a non-volatile nature. This arrangement enables one to effect a rough though not very satisfactory separation of the species.

Thus the following species *F. viride*, Ag. 81 ; *F. solani* var. *minus*, Ag. 83 ; *F. lini*, Ag. 60 ; *F. Martii*, Ag. 77 ; *F.* species (from potato), Ag. 80, and *F. dianthi*, Ag. 59, seem to form a group having the following characteristics :—None of them produces any appreciable amount of " non-volatile acids," while all produce relatively large amounts of " volatile acids." This is, of course, in direct contrast to the majority of species of *Aspergillus*, in which the acidity produced is almost entirely of the " non-volatile acid " type. The nature of this volatile acid has not been investigated, but if it were desired to do so it is obvious that the two species, Ag. 81 and Ag. 83, would be the most suitable for the purpose, since the " carbon in volatile acids " is 0·198 and 0·212 gm. respectively, corresponding to yields of 8·0 and 7·2 per cent. of the sugar fermented. Each of the five species in this sub-group also gives rise to moderate amounts of " carbon unaccounted for," though in no case is this very large, since the maximum is 0·411 gm. with *F.* species, Ag. 80, corresponding to 18 per cent. of the sugar fermented.

Further indication that these species may be regarded as a separate sub-group is the fact that while all of them give rise to larger weights of mycelium than the majority of the other species of *Fusarium* investigated, the amount of glucose metabolized by them is small. The amount of " volatile neutral compounds " formed by these five species is also very much less than the average for the remainder, and their respiration coefficients are very much lower than those of the other species.

Another sub-group might be formed of the following species :—*F. uncinatum*, Ag. 75 ; *F. trichothecioides*, Ag. 73 ; *F. tubercularioides*, Ag. 72 ; *F. cœruleum*, Ag. 65 ;

F. salicis, Ag. 69; and *F. javanicum*, Ag. 78, having the following characteristics :—The acidity produced by these species is, in contrast to the first sub-group, largely of the non-volatile acid type, and in this respect they resemble the *Aspergilli* more than any other species of *Fusarium*. The amount of titratable acidity or of non-volatile acids is, however, not very large, although it is sufficiently so to mark these species as being somewhat different from the remainder of the species of *Fusarium* investigated. All species in this group produce large amounts of volatile neutral compounds, and all of them have very large respiration coefficients. It will be noted that with almost all the species in this group, and also with *F. falcatum*, Ag. 82; *F. avenaceum*, Ag. 76; and *F. sporotrichoides*, Ag. 58, which may be regarded as the connecting link between this group and the remainder of the species of *Fusarium*, there is in every case a very marked difference between the percentage of residual glucose as estimated by alkaline iodine and by the Shaffer-Hartmann method. It is very probable that this is due to the characteristic which we have shown these species of *Fusarium* to possess, of reducing the sodium nitrate present in the Czapek-Dox medium to ammonia, which, in presence of alkaline iodine, forms iodine-substituted nitrogen compounds (probably nitrogen tri-iodide), hence giving results for glucose present which are far too high.

The remainder of the species of *Fusarium* which are given in Table II have no very special characteristics, except that they all give rise to very large amounts of volatile neutral compounds, and all have very high respiration coefficients.

Summary.

A quantitative examination has been made by the carbon balance sheet method, described in Part II, of the types of products formed from glucose by 23 species of *Fusarium*. These carbon balance sheets are presented in two tables from which a rough biochemical classification of the *Fusaria* can be made, but this is not nearly so satisfactory as that evolved for the *Aspergillus* group.

The main biochemical characteristic of species of *Fusarium* is that, like the *Saccharomyces*, they give rise to large amounts of alcohol from glucose. It is suggested that, since many species of *Fusarium* grow readily on waste vegetable materials, it may be possible to solve the problem of producing alcohol from waste vegetable matter by the use of a selected species of *Fusarium*.