Midland Counties Institute of Brewing.

MEETING HELD ON THURSDAY, 13th DECEMBER, 1900, AT THE GRAND HOTEL, BIRMINGHAM.

Mr. W. W. Butler (President) in the Chair.

The President, in opening the proceedings, said it was quite unnecessary for him to introduce to them the gentleman who had kindly consented to read a paper, Professor Adrian Brown. He was, as they well knew, head of the Brewing School established at the University, and the members would remember that they were very much indebted to him at the last meeting for reading two papers.

The following paper was read and discussed:—

The Heat of Fermentation.

By ADRIAN J. BROWN, F.I.C., Professor at the British School of Malting and Brewing, University of Birmingham.

(The paper read to the meeting was a more popular account of the subject.)

THE main object of this paper is to describe some experiments conducted by the author with a view to measure the amount of heat liberated during alcoholic fermentation; but previous to doing so it may be useful to review what is known concerning the heat changes taking place during fermentation, as there is little or nothing to be found on the subject in either chemical text books or technical works on brewing.
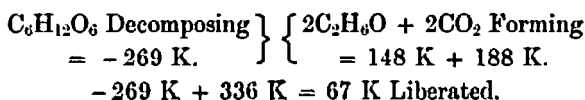
The fact that heat is liberated during alcoholic fermentation has been common knowledge for ages past, but the true explanation of the cause of this liberation of heat was not found until quite recent times. Indeed, until very considerable knowledge was gained, not only of the chemical changes taking place during fermentation, but also of the thermo-chemistry of the elements and compounds taking part in these changes, no advance could be made in this direction.

The mere knowledge that the chemical change taking place during fermentation is a breaking down of the sugar molecule into alcohol and carbonic dioxide, does not in itself help much in understanding why heat is evolved during the process. It tells us it is not due to ordinary oxidation, and suggests that it must be due in some way to the rearrangement of the constituent atoms of the sugar, but does not carry us farther. The aid of thermo-chemistry has to be called in to demonstrate satisfactorily the true cause of the phenomenon.

So far as the author is aware, Berthelot was the first to do this (*Ann. Chem. et Phys.*, 4th series, t. vi, 399). This chemist (taking as his basis the equation expressing the fermentation of dextrose, $C_6H_{12}O_6 = 2\ C_2H_6O + 2\ CO_2$) found by direct experiment that 1 gram-molecule of dextrose on combustion liberated 713,000 calories,[*] but that the sole combustible product of fermentation derived from it, viz., two molecules of alcohol,

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[*] Throughout this paper "calory" represents the heat required to raise 1 gram water 1° C.; "K" represents the heat required to raise 1000 grams water 1° C.

liberated only 642,000 calories when burned. That is to say, the constituent atoms of a gram-molecule of dextrose, arranged as they are in that sugar, yield 71,000 more units of heat on combustion than when arranged as two molecules of alcohol and two molecules of carbon dioxide. This evidently indicates that the rearrangement of the atoms composing the dextrose molecule during fermentation must be accompanied by the liberation of energy, for otherwise there is no means of accounting for the difference between the heats of combustion of dextrose and its fermentation products, but how it comes about is not very evident from Berthelot's demonstration.

A clearer exposition of the reason has, however, been put forward by C. v. Rechenberg (*J. pr. Chem.*, **22**, 1–45). This author found by direct experiment that the heat of combustion of a gram-molecule of dextrose was 709 K,[*] and then showed that during the combustion of dextrose the action may be resolved into two stages—

$$(\alpha)\ C_6 + H_{12} + O_6 = C_6 + H_{12} + O_6 \atop (\beta)\ C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O \Big\} = 709\ K.$$

If now the heat evolved in such an action as β is calculated by means of the known heats of combustion of the elements concerned, the following result is obtained :—

$$6C = 6CO_2 = 564\ K.$$
$$12H = 6H_2O = 414\ ,,$$
$$6O = \qquad\qquad ,,$$
$$\overline{978\ K.}$$

This shows that if the gram-molecule of dextrose was merely a mechanical mixture of free C, H, and O molecules, 978 K would be liberated by its combustion; but it is a chemical compound, and has been found by direct experiment to liberate 709 K when burned. The difference, 269 K, must therefore be the heat of formation of dextrose, that is to say, 269 K are liberated during the building up of the sugar molecule from its elements. Hence dextrose is an exothermic compound.

Now the liberation of heat by the breaking down of the molecule of an exothermic body does not at first sight appear possible, for exothermic compounds absorb heat during decomposition into their elements.

* *Ante*, p. 93.

However, although dextrose is *exothermic* with regard to its constituent elements, it is *endothermic* with regard to its fermentation products. Thus the calculated heat of formation of alcohol is 74 K for each gram-molecule, and the heat of formation of carbon dioxide found by direct experiment is 94 K. Under these circumstances, the following equation represents the heat changes taking place during fermentation :—

$$C_6H_{12}O_6 \text{ Decomposing} \begin{array}{l} \\ = -269 \text{ K.} \end{array} \Big\} \Big\{ \begin{array}{l} 2C_2H_6O + 2CO_2 \text{ Forming} \\ = 148 \text{ K} + 188 \text{ K.} \end{array}$$
$$-269 \text{ K} + 336 \text{ K} = 67 \text{ K Liberated.}$$

So the heat of formation of alcohol and carbon dioxide liberated during fermentation, together exceed the heat absorbed during the decomposition of the exothermic sugar, and the excess represents the heat of fermentation.

Another view of the same heat changes may be taken. When the sugar molecule is built up by the processes of plant assimilation, its constituent atoms are not arranged in such a manner as to exhaust their potential energy of combination—there is a store of potential energy remaining that is capable of liberation by a different arrangement of the atoms. This rearrangement is brought about by the zymase of the yeast-cell, with a consequent evolution of heat.

From a thermo-chemical point of view, the fermentable sugars may therefore be regarded as akin to explosive substances, such as nitroglycerin.

Berthelot has also calculated the heat of fermentation of dextrose in a similar manner to Rechenberg, but has based his calculations on what he believes to be more accurate determinations of heats of combustion. He arrives at the following result :—

$$\frac{C_6H_{12}O_6}{-300K.} = \frac{2C_2H_6O + 2CO_2}{144\cdot8 + 188\cdot6} = 33 \text{ K.}$$
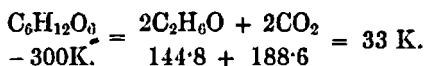
He has also carried his calculation further in an endeavour to estimate more accurately the amount of heat liberated. With this object in view he has corrected for the formation of the minor products of fermentation, glycerin and succinic acid, according to the numbers expressed by Pasteur's equation, thus :—

$$171 \cdot 7 \text{ dextrose into } C_2H_6O + CO_2 \qquad = 31 \cdot 47 \text{ K.}$$
$$8 \cdot 3 \quad \text{,,} \qquad \text{,, glycerin and succinic acid } = 00 \cdot 60 \text{ ,,}$$

$$\text{Total heat of fermentation } = \overline{32 \cdot 07} \text{ K.}$$

Attempts at such accuracy as this, however, appear superfluous when the probable error, introduced by the method of calculation adopted, is so very large. As an instance, Berthelot, when using heats of combustion differing a little from those employed by Rechenberg, arrived at a result about 50 per cent. lower than the result found by that author. But the errors to which calculated heat determinations, such as these, are liable, need only passing reference here, as they have already been fully discussed by H. Brown and Pickering (*J. Chem. Soc.*, 1897, **71**, 783) in their paper on "The Thermochemistry of Carbohydrate Hydrolysis."

Probably Berthelot's indirect measure of the heat of fermentation is an approach to the truth, but it cannot be accepted with any confidence. Reliable measures can only be obtained by direct experiment ; but, on the other hand, the experimental difficulties to be overcome in order to obtain them are very great. It is probably for this reason that only one investigator, Bouffard, has, so far as the author is aware, attempted the task.

In the *Compt. Rend.* (1895, **121**, 357) this chemist describes the results of a series of six experiments conducted on fermenting grape juice in a Berthelot calorimeter. The volume of grape juice he employed was 1 litre, and a further quantity of dextrose was added to the liquid in order to increase the amount of fermentable sugar. The experiments appear to have been planned and carried out with great skill and care, and the final results arrived at are in close agreement.

Bouffard's experiments indicate that the heat of fermentation of dextrose is 23·5 K, or about two-thirds of the calculated heat found by Berthelot ; but he advances his conclusion with some diffidence, and suggests that the true heat of fermentation probably lies somewhere between 23·5 K and the 32·07 K of Berthelot. However this may be, the author does not share with Bouffard the feeling that there is any reason to mistrust his results merely because they do not agree more closely with Berthelot's. Nevertheless, it is very desirable to get additional experimental determinations, and, if possible, procure them by a different means to that employed by Bouffard.

Among the many difficulties met with when attempting to measure the heat of fermentation in a calorimeter, the greatest is due to the powerful influence of external temperature, owing to the comparatively slow evolution of the heat and the small volume of liquid that must be used. In Bouffard's experiments the correction for this influence alone amounted to about 25 per cent. of the heat measured.

Now, if the volume of the liquid experimented on could be increased considerably, it is evident this influence would be much diminished, for it is through its surface that the heat exchanges of a mass take place, and the proportion of surface to mass diminishes as the mass increases.

Taking this into consideration it appeared to the author that it might be possible to make use of a brewery fermentation to measure the heat liberated during fermentation, for in a brewery a volume of fermentable liquid can be used large enough to reduce the influence of external temperature to a minor factor.

No doubt at first thought the idea of calorimetric measurements being made in a brewer's fermenting vessel does not appear to promise accurate results ; but there are some forms of fermenting vessels which are very well adapted for use as calorimeters, and the author believes there is much to be said in favour of their employment for this purpose.

The particular fermenting vessel used in the author's experiments was rectangular, its length being 9 feet, breadth 4 feet, and depth 6 feet. It was constructed of thin copper, and was cased with wood about 1 inch in thickness. The top of the fermenting vessel was open, but when an experiment was being conducted, the surface of the fermenting malt-wort it contained was covered with about 12 inches of the persistent "head" or foam which accompanies active fermentation under such circumstances, and this furnishes an almost perfect protection from heat radiation. Used as a calorimeter, therefore, this fermenting square was well protected from excessive loss through heat radiation, and the usual correction for its thermal capacity could be made as the weight of the copper composing it was known. During an experiment, no form of mechanical stirrer was found necessary, as the brisk evolution of gas due to fermentation did the work most effectually.

Although, by employing a well-protected calorimeter of very large

size such as the one described, the influence of the outer temperature is
diminished very considerably, its effect is still sufficient to require
some means of correction.   The means employed will be described
later on.

In order to utilise fermenting malt-wort for the purpose of mea-
suring the heat of fermentation, it is necessary—(1), to ascertain the
rise in temperature of the fermenting wort during an interval of time ;
(2), to ascertain the weight of sugar decomposed during this time
interval ; and (3), to ascertain the specific heat of the fermenting wort.

The rise in temperature is easily determined by means of a Beck-
mann differential thermometer plunged directly in the fermenting
liquid.   But the accurate estimation of the sugar fermented is more
difficult.   The use of the polarimeter for the purpose naturally
suggests itself, and it would act very efficiently if the liquid experi-
mented on contained only one fermentable sugar.   But in malt wort,
although most of the contained sugar is maltose, small quantities of
dextrose and levulose are also present, and as the optical properties of
these sugars differ very widely from those of maltose, very small
quantities, when fermented with it, cause an appreciable error if the
polarimeter is relied on to determine the amount of sugar fermented.

A somewhat similar objection applies to the estimation of the
fermented sugar by means of copper-reduction determinations, for the
reducing power of maltose differs very considerably from that of dex-
trose or levulose.

The method the author adopted to arrive at the amount of sugar
fermented, was based on the loss in specific gravity of the solution
during fermentation.

If samples of the fermenting wort, taken at the beginning and end of
an experiment, are freed from alcohol and the specific gravity of each
is determined, the difference between these two weights represents the
solution weight of the sugar decomposed.   If this difference is divided
by the known solution weight of maltose, the weight of the dry sugar
fermented is obtained.   It is true that the solution weights of dextrose
and levulose differ somewhat from that of maltose, and that a small
error must be introduced by this method of working, but it is inappre-
ciable in actual practice, the differences in the solution weights of these
sugars being very small, unlike the large differences in their reducing
and rotatory powers.

The specific heat of the fermenting wort was ascertained by the method of mixture; it varies but little and may usually be taken as 0·968.

Experimental Method.

When the wort in the copper square was in a vigorous state of fermentation and had reached a suitable temperature for experiment, the heat of the room was noted, and a reading was taken of the temperature of the fermenting wort with the Beckmann thermometer. Immediately afterwards a sample of the wort was withdrawn from the square and shaken in a closed vessel with a little salicylic acid to arrest fermentation. A measured volume of this wort was then heated to expel the contained alcohol, cooled, and made up to the original volume. Its specific gravity was then determined in a pyknometer. This concluded the first stage of the experiment.

Afterwards, when the temperature of the wort in the fermenting vessel had risen from one to two degrees centigrade, a repetition of the first series of determinations was made, the relative order and time of execution being strictly adhered to, in order that—as the experimental method employed was essentially a differential one—every advantage might be taken of the way such a method permits many experimental errors to correct themselves.

The details of an experiment and calculation are given below.

Experiment II.

	1st series of observations.	2nd series of observations.
1. Time	12.0 noon	5·45
2. Temperature of room.....................	17·0°	19·0°
3. Approximate temperature of fermenting wort	17·2°	19·2°
4. Reading of differential thermometer...	4·960°	2·955°
5. Specific gravity of wort after separation of alcohol...	1053·624	1047·173
6. Approximate maltose in wort by CuO determinations..........................	8·776 per 100 c.c.	6·972 per 100 c.c.
7. Specific gravity of wort before separation of alcohol	1051·399	1043·596

The solution factor for maltose at the mean concentration indicated by the CuO determinations (6) is 0·393,* therefore

$$\frac{1053\cdot624 - 1047\cdot173}{3\cdot93} = 1\cdot6415 \text{ maltose fermented in 100 c.c. wort.}$$

But 4 per cent. of this sugar when fermented is converted into glycerin, which has a solution factor for dilute solutions of 0·233; hence $1\cdot6415 + 0\cdot039 = 1\cdot6805$ corrected amount of maltose fermented.

$4\cdot960° - 2\cdot955° = 2\cdot005°$, the rise in temperature during the experiment; but the wort has a specific heat of 0·9678, therefore $2\cdot005° \times 0\cdot9678 = 1\cdot9404°$, the true rise in heat calculated for 100 c.c. water. But this amount also requires correction for the heat capacity of the copper fermenting vessel. This was determined to be equal to 0·68 gram water on the 100-gram volume on which the calculation is based, hence

$$\frac{1\cdot9404 \times 100\cdot68}{100} = 1\cdot9536°$$

rise in temperature during fermentation. Therefore

$$\frac{1\cdot9536 \times 100}{1\cdot6805} = 116\cdot2 \text{ cals.}$$

liberated by the fermentation of 1 gram maltose.

But carbon dioxide issues from the fermenting wort saturated with water vapour, the evaporation of which carries off heat. Therefore as 0·464 gram CO_2 is evolved during the decomposition of 1 gram maltose, this, at the temperature of experiment, contained 0·004 gram OH_2, the evaporation of which has absorbed

$$\frac{10000 \times 0\cdot004}{18} = 2\cdot2 \text{ cals.}$$

Hence $116\cdot2 + 2\cdot2$ cals. $= 118\cdot4$ cals. evolved during the fermentation of 1 gram sugar.

In this calculation the weight of fermented sugar has been determined from its solution weight, but another method can be adopted to check the result.

It has been recognised by H. T. Brown (*J. Chem. Soc.*, 1873, 981), Stern (*J. Soc. Chem. Ind.*, 1900, **19**, 127), the author, and others that during alcoholic fermentation the change in volume of the fermenting liquid is exceedingly small—so small indeed that it may be

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* See Brown, Morris, and Millar, "Examination of the Products of Starch Hydrolysis by Diastase," *Journ. Chem. Soc.*, 1897, **71**, p. 77.

neglected in most cases. Under these circumstances it is evident that the difference between the specific gravity of a liquid before and after fermentation must represent very closely the weight of the carbon dioxide evolved, for carbon dioxide is the only matter separated from the mass of the solution during fermentation.*

In the experiment just described, the specific gravity of the wort at the commencement of the experiment was 1051·399, and at its termination 1043·596.

Therefore the weights of 100 c.c. of each are—

$$\begin{array}{r} 105\cdot1399 \text{ grams.} \\ 104\cdot3596 \text{ ,,} \\ \hline \text{Difference} = \quad 0\cdot7803 \text{ ,,} \end{array}$$

and the difference 0·7803 gram is the weight of the CO_2 evolved from 100 c.c. during fermentation.

According to Pasteur, 0·464 gram CO_2 is evolved during the fermentation of 1 gram of a $C_{12}H_{22}O_{11}$ sugar, therefore $\dfrac{0\cdot7803}{0\cdot464} = 1\cdot6817$ grams maltose fermented in the experiment.

Using this determination of the maltose as in the previous calculation, we have $\dfrac{1\cdot9536 \times 100}{1\cdot6817} = 116\cdot17$ cals., which, corrected for water vapour, gives 118·39 cals., in place of the 118·4 cals. found by the first method.†

The following table gives the results of four heat determinations calculated in the manner first described, and the mean temperatures of the wort and the room during the experiment:—

		Mean temp. of wort.	Mean temp. of room.	Calories found.
No. 1	......	16·8	17·5	121·9
,, 2	......	18·2	18·0	118·4
,, 3	......	17·0	14·7	116·1
,, 4	......	19·3	13·7	111·7

So far no allowance for loss of heat by "cooling" has been made, and in the above table the effects of cooling are very apparent if the

* Although this conclusion is so very evident, I do not recollect having seen it noticed before.

† The determinations are given as observed, but the *very close* agreement is probably accidental.

figures representing the mean temperatures of the experiments, and the external temperatures, are compared with the calories found. In the first experiment when the mean external temperature exceeded that of the fermenting wort by 0·7°, 121·9 calories were measured, and in the fourth experiment when the external temperature was 5·6° lower than the wort, only 111·7 calories. Experiments 3 and 4 were conducted purposely under circumstances when the differences of internal and external temperature were considerable in order to measure and note the effect; but experiments 1 and 2, in which the difference is very small, should give results closely approaching the truth. In No. 2, where the difference was only 0·2, the influence would perhaps be within the errors of experiment. However, it has been deemed advisable to plot a curve of all the experiments and correct by this means. In this way the author finds that the heat of fermentation of maltose is 119·2 calories.

In order to make this measure of 119·2 calories comparable with Bouffard's, which is calculated on the gram-molecule of dextrose, it has also been calculated on the same basis, with the result that 21·4 K* are found by the author as against Bouffard's 23·1 K. But in Bouffard's calculation he assumed that 0·4 K was carried off by the carbon dioxide evolved, a correction the author has not adopted, therefore the two results stand for comparison—

<div style="text-align:center">

Bouffard .... 23·1 K.

Author .... 21·4 „

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The agreement is close considering the very different methods by which the results were obtained; but it must be borne in mind it is very probable that the agreement is not so close as the figures seem to imply. Bouffard experimented with dextrose, a sugar which ferments directly; the author experimented with maltose, a sugar which is hydrolised previous to fermentation. It is known that cane-sugar, a sugar of somewhat similar constitution to maltose, liberates heat equal to 3·8 K on hydrolysis. (H. Brown and Pickering, *J. Chem. Soc.*, 1897, **71**, 792.) It is not known at present what heat changes accompany the hydrolysis of maltose, but it is probable heat is liberated to a somewhat similar extent. If so it must of course be included in the author's measure of the heat of fermentation of maltose. The present state of knowledge, however, does not admit the expression of any

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* As the molecular weight of maltose is 342, its true K is 40·7.

definite opinion. The author is aware that his method of attempting to measure the heat of fermentation, is, in common with Bouffard's, open to criticism, and perhaps the results of neither express the truth very exactly; but they both have the merit of supplying results derived from direct experiment when such are needed.

The author hopes that this description of his experiments may assist in calling attention to the unsatisfactory state of our knowledge concerning the heat changes taking place during fermentation, and to an extensive field open to investigation in this direction.

An interesting discussion followed the reading of Professor Brown's paper, in which the President, Messrs F. L. Talbot, Russell, G. R. Burdass, and E. Evans took part. The report of this discussion is, however, omitted, for it could not be followed with advantage as it was based on a different treatment of the subject to the one given above.

The Annual Banquet had been fixed for the 31st January. In consequence of the lamented death, however, of Her Most Gracious Majesty Queen Victoria, it was postponed.

THE EIGHTH ANNUAL MEETING, HELD ON THURSDAY, 31st JANUARY, 1901, AT THE GRAND HOTEL, BIRMINGHAM.

Mr. W. W. BUTLER (President) in the Chair.

The Annual Report and accounts were approved and the officers and Committee re-elected.

THE ANNUAL REPORT.

The Seventh Annual Report contained an account of the proceedings at the Seventh Annual General Meeting and Banquet.

A statement was given showing the membership of the Institute since its formation.

A list of the papers read before the Institute was given, and the Committee expressed their thanks to the various authors.

The Report was dated 4th January, 1901.