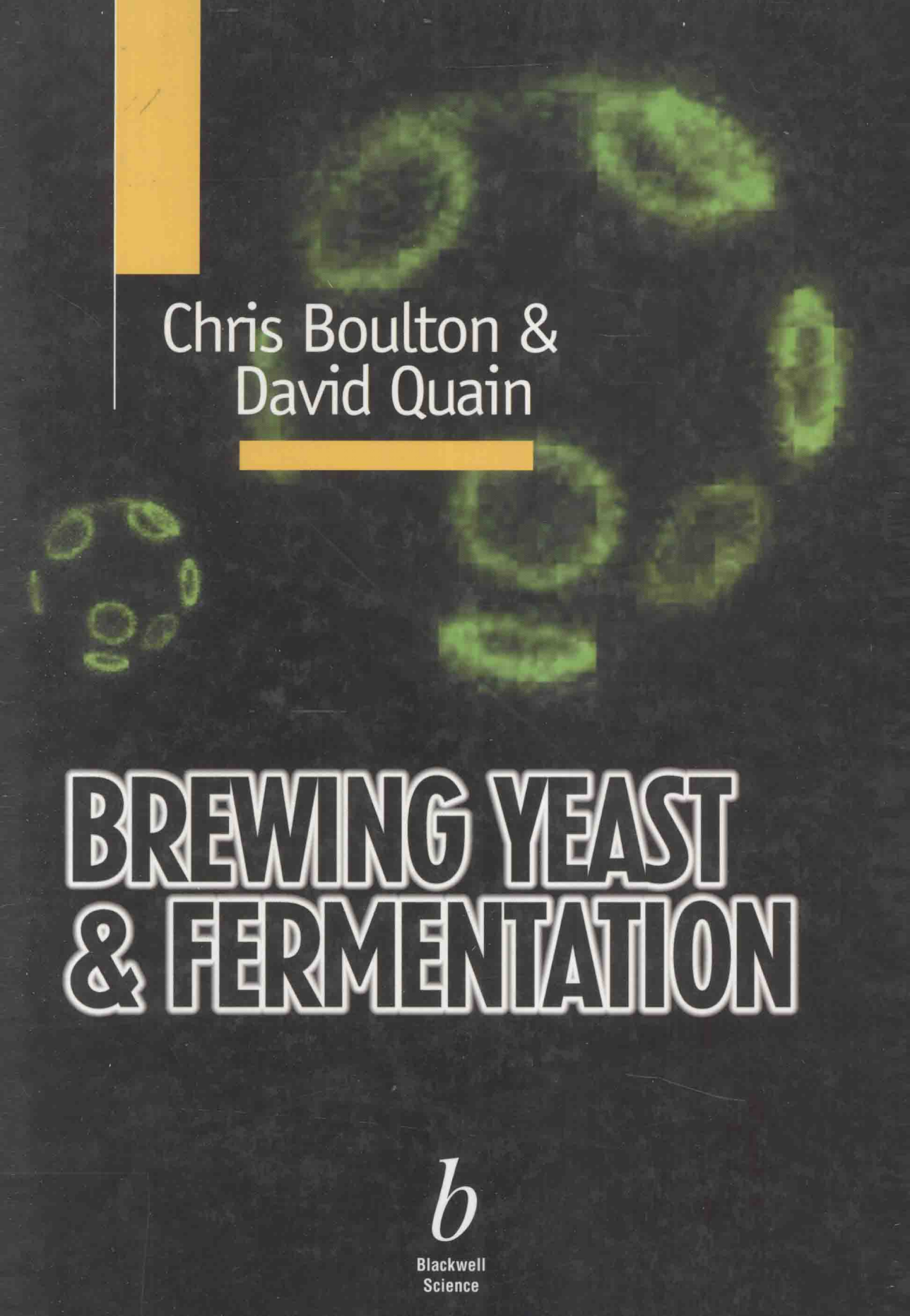

Chris Boulton &
David Quain

BREWING YEAST & FERMENTATION

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Brewing Yeast and Fermentation

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Preface

As is perhaps suggested by the title, this book was written with the intention of filling two perceived gaps in the current literature. In the first instance the authors hope that they have provided a comprehensive description of brewery fermentation, both traditional and modern. Secondly, to underpin this, a detailed review is presented of current understanding of those aspects of the biochemistry and genetics of yeast, which impact on brewery fermentation.

Of course, both of these related topics have received considerable attention elsewhere. For example, the multi-volume texts, *Brewing Science* (Pollock, 1979) and *Malting and Brewing Science* (Hough *et al.*, 1981; Briggs *et al.*, 1981), provide detailed descriptions of the brewing process in its entirety. Both of these are effectively standard works of reference and are highly regarded. However, both now have a vintage of some twenty years. During the period since their publication much has happened to the brewing industry as it has perforce adapted to changes in the global market place. Economic and social forces coupled with developments in science and technology have resulted in profound changes in the practice of fermentation and the range of beers produced. Hopefully these developments, together with a full account of the still much used traditional processes, are captured in this book.

The scientific literature devoted to yeast belonging to the genus *Saccharomyces* is extensive and diverse. This is fitting, since strains of this organism are of paramount importance in the biotechnological processes of brewing, wine making and baking. In addition, the type species, *Saccharomyces cerevisiae*, has been much employed worldwide in research laboratories as *the* model eukaryotic cell. Indeed, the publication of the sequence of the *S. cerevisiae* genome in April 1996 was a milestone in late twentieth-century biology. The plethora of primary literature concerning the *Saccharomyces* and other yeast genera, is reviewed in depth elsewhere. Most notably in the consistently excellent multi-authored series, *The Yeasts* (Rose & Harrison, 1987 *et seq.*; Rose *et al.*, 1995). However, it is perhaps true that the mainstream of yeast research has dwelt little on the elucidation of the biochemical activities of yeast in the complex systems associated with growth on poorly characterised media such as brewers' wort, under conditions of transient aerobiosis. Indeed in some instances it has perhaps been assumed that yeast metabolism under the conditions of a brewery fermentation are more similar to those which pertain to aerobic growth on a defined medium than is, in fact, the case. These issues, together with a review of the misunderstood but steadfastly important subject of brewing microbiology, are addressed in this present work.

The foregoing discussion raises the question as to who is the target audience for this

volume? The answer is everyone who has an interest, either as a seasoned professional or student, of the noble art of brewing. In addition, we hope that the wider academic community of zymologists will also find items of interest. Much of the content of the book is devoted to fermentation as practised by large scale commercial brewers, however, we have endeavoured to include material of interest to those who operate the time-honoured traditional processes.

The authors are associated with the UK brewing industry. We have provided an international perspective by highlighting aspects of the fermentation of beers which are peculiar to individual countries. Of course, the promulgation of developments in brewing science, in common with any other academic discipline, has no regard for national boundaries and we humbly dedicate this book to the spirit of that tradition.

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Chris Boulton would like to thank many friends within the brewing industry and the academic community who have contributed both directly and indirectly to this project. In particular, I acknowledge the help of colleagues, past and present, with whom I have had the privilege of working with at Bass Brewers. Without their collective wisdom, this book would not have been possible. To name individuals is always invidious, however, I acknowledge Dr Peter Large and Professor Colin Ratledge of Hull University for teaching me to appreciate the delights of biochemistry and yeast physiology. Thanks to Professor Charlie Bamforth of Davis University, California who was responsible for diverting my attention from obese yeasts to the alcoholic varieties. I thank Dr Vincent Clutterbuck, Dr Sean Durnin, Dr Stuart Molzahn, David Lummis, Preston Besford and particularly Wendy Box for numerous fruitful discussions and collaborations. Special thanks to my co-author, David Quain, for perseverance and inspiration. Finally, I am indebted to my wife, Wendy, for patience and understanding above and beyond the call of duty; without her it would not have happened.

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I would also like to formally acknowledge those who made a difference to my career in a variety of ways. These include my Mother and lifelong friends, Dave Fenton and Alistair Stewart. Looking back over the last 30 years, a number of people 'made a difference' and provided direction and support. These include Dr John Hudson, Professor Anna MacLeod, Ted Hickman, Dr Pat Thurston, the late (and sadly missed) Dr Andrew Goodey, Professor Graham Stewart, Dr Philip Meaden, Dr Jim Haslam, Dr Tony Portno, Dr Fintan Walton, Roger Putman, Dr Harry White, Professor Charlie Bamforth, and especially Dr Stuart Molzahn.

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Contents

1 Beer and brewing

1.1 Introduction

In order to delineate the boundaries of brewery fermentation it is necessary to define the product of the process, beer. In particular, how is beer distinguished from other beverages, which are produced by fermentation. The etymology of the word gives little clue other than inferring a certain universality, it being derived from the Latin *bibere*, to drink. The *Shorter Oxford Dictionary* describes beer as 'alcoholic liquor obtained by the fermentation of malt (or other saccharin substance) flavoured with hops or other bitters'. For the purposes of this book this definition is too proscriptive. Beer is now used as a generic term for a multitude of beverages of widely differing appearance and flavour and produced from a variety of raw materials. Whilst it is true that in most cases malted barley provides the major source of fermentable sugars, many other sources of extract may be used. Beers may or may not contain hops or other bittering substances. A whole range of other flavourings may be added including fruits, spices and various plant extracts. In some cases beers may not even contain alcohol.

Another way of defining beer is to consult the relevant legislation. In fact. this is also relatively unhelpful in that it has usually been framed with the specific intent of establishing standards for alcohol content, setting up a framework for collecting taxes and as a means of controlling the activities of manufacturers and consumers. Frequently the product itself is relatively undefined. Thus, in the United Kingdom, the Alcoholic Liquor Duties Act (Her Majesty's Stationery Office, 1979) describes beer as:

> 'including ale, porter, stout and any other description of beer, and any liquor which is made or sold as a description of beer or as a substitute for beer and which on analysis of a sample thereof at any time is found to be of a strength exceeding 2° of proof [1.2% ethanol v/v].'

The same Act has specific categories for other alcoholic beverages such as wine, cider/perry and spirits. These are more obviously distinctive in that manufacture involves distillation, or fermentation of a medium largely obtained from a single specific raw material not associated with brewing such as grape or apple juice. However, the Act does include another catch-all category, 'Made Wine', which is defined as

> 'any liquor obtained from the alcoholic fermentation of any substance, or by mixing a liquor so obtained or derived from a liquor so obtained with any other liquor or substance but does not include wine, beer, black beer, spirits or cider'.

Even allowing for the somewhat impenetrable prose it is apparent that legal distinctions are made in the United Kingdom between beer and other beverages, which may at the very least, be open to interpretation elsewhere.

Most other countries have a legal definition of beer, which is similar to and equally as vague as that of the United Kingdom. A notable exception is Germany where a very distinct view is taken as to what is and what is not a beer. Germany has very stringent laws, which define precisely what may be labelled as beer and the nature of the materials that may be used during its manufacture. The *Reinheitsgebot* or beer purity laws are based on legislation introduced into Bavaria, in 1493, by Duke Albrecht IV. The original law stipulated that beer could be made only from malted barley, hops and water; yeast was not included since at that time it was undiscovered (see Section 1.2). Subsequent manifestations of the laws included this vital ingredient when it was observed that more consistent fermentations were obtained when inoculated with the crop harvested from the previous fermentation. The purity laws are still in operation and prohibit the use, in Germany, of many ingredients and process aids which are legally acceptable elsewhere (Narziss, 1984).

For the purposes of this book and in order to maintain a proper international perspective, beer is simply defined as a beverage produced by fermentation of an aqueous medium which contains sugars derived mainly from cereals. The fermentation step is catalysed principally by yeast. The latter may be a pure monoculture, or a mixture of yeast strains, with or without the involvement of bacteria.

Having defined the product, it is necessary to consider the scope of the fermentation process used in its production. The range of fermentation practices that may be encountered differ mainly in terms of scale and sophistication. At its simplest the process may be little more than a domestic operation producing beer in volumes sufficient for family consumption. Such undertakings tend to use procedures which are relatively undefined and have been developed by empirical observation and practice. At the other end of the scale, modern industrial breweries may have fermentation capacities of several million litres and use a process which approaches pharmaceutical standards in terms of control, hygiene and use of defined microbial cultures. Intermediate to these extremes is a continuum that ranges from purely traditional breweries, which may produce beer in considerable volume but also use empirically derived practices and equipment which has changed little over many centuries, through to the 'modernised' traditional brewery. In the latter case the basic process is unchanged and perhaps still relatively undefined but new plant has been installed, usually with greater capacity and improved hygienic properties (see Chapter 5).

Irrespective of the scale of operation, brewing has long been – and with few notable exceptions – continues to be practised as a batch process. It consists of three principal stages. First, wort production in which raw materials are treated to produce an aqueous medium rich in sugars and various other yeast nutrients. Second, fermentation, the stage in which certain components of the wort are assimilated by yeast cells to produce ethanol, carbon dioxide and a multitude of other metabolic products, which collectively form beer. Third, post-fermentation processing in which the immature beer is rendered into a form in which it is considered suitable for drinking.

Although this book concentrates on the fermentation stage of brewing, it is necessary to include some discussion of other parts of the process (see Chapter 2). With regard to the initial stages, fermentation performance is much influenced by wort composition. Since the latter is affected by the methods and materials used in its

production, it is necessary to provide here a description of the processes involved. After primary fermentation is completed, various procedures may be carried out which require further activity by yeast (or other micro-organisms). These processes are grouped under the heading of secondary fermentation and obviously merit discussion (see Section 6.9).

Whilst it is true that the vast majority of brewers produce beer by batch fermentation there is at present a renaissance of interest in continuous processes. This follows an earlier foray, which occurred during the 1960s and 1970s. At that time continuous fermentation was seen by many of the larger companies as a potential cost-effective method for satisfying the need for highly efficient and high-productivity brewing. During this period several brewers invested in large-scale continuous fermentation plant. Unfortunately, practical experience failed to live up to the theoretical promise, and, with a few exceptions, application at production scale was largely discontinued by the 1980s. In the last decade, however, there has been a resurgence of interest in continuous brewing fermentation largely because of the development of new systems which employ immobilised yeast (see Sections 5.6 and 5.7).

Parallel to a discussion of the practice of brewery fermentation is a consideration of the biochemical changes, that encompass the conversion of wort to beer (see Chapter 3). These are as fascinating as they are complex. Thus, the growth and metabolism of the yeast, *Saccharomyces cerevisiae*, has been subject to intensive scrutiny over a number of years and it is now one of the best characterised of all eukaryotic cells. Indeed many of the groundbreaking discoveries on which modern biochemistry is based were founded on research performed with brewers' or bakers' yeast. However, much of this work has perforce studied metabolism under carefully controlled conditions; usually aerobiosis, frequently non-growth, growth in continuous culture on defined media, or batch growth on defined media. Of all of these batch growth, even on a defined medium, is the most difficult to characterise since conditions are in a constant state of flux and a steady state is never achieved.

This difficulty applies to a batch brewery fermentation; however, superimposed on this are several other layers of complexity. The growth medium, wort, is a natural product that contains a multitude of components, many yet to be identified and fluctuating in concentration from batch to batch. Several of the constituents, in isolation, are known to exert positive and negative influences on yeast metabolism; however, in combination they may interact to produce new and totally unexpected synergistic and antagonistic effects. This situation is further complicated by the role of oxygen in brewery fermentations. It is often assumed by the uninitiated that a beer fermentation is an entirely anaerobic process but, in fact, this is not so. For prolonged growth under anaerobic conditions, brewing yeast must have sources of unsaturated fatty acids and sterols. Some of this requirement may be satisfied by assimilation from wort but most has to be synthesised, *de novo*, and this requires molecular oxygen (see Section 3.5). Thus, at the beginning of fermentation, wort is provided with a single dose of oxygen, which is utilised by yeast during the first few hours of fermentation producing a transition from aerobiosis to anaerobiosis. This unsteady state, coupled with the other changes associated with growth and metabolism, has far-reaching effects on yeast physiology, many of which remain to be fully characterised.

The biochemistry of brewery fermentation is further complicated by virtue of the fact that vessels are not usually provided with forced agitation and neither is there any standardisation with respect to vessel configuration. Mixing of the fermenting wort to encourage adequate mass transfer of metabolites and dispersion of yeast cells is reliant upon natural convection currents brought about by the combined effects of escaping gaseous carbon dioxide and localised differences in fluid density due to differential cooling. During primary fermentation this natural agitation may be vigorous and probably sufficient to ensure homogeneity of the contents of the fermenter. However, when the yeast is relatively quiescent, at the outset and in the later stages, considerable stratification may be evident. This heterogeneity results in individual cells within the yeast population being exposed to widely differing environments depending on their particular location within the vessel. In this respect, physical parameters such as hydrostatic pressure and possibly temperature are important, particularly where very deep vessels are employed (see Section 5.1).

Intimately related to the biochemistry of fermentation is a consideration of the yeast used in the process. This topic may be divided into two areas of discussion. First, a description of the general biology and classification of brewing yeast. This includes a description of cellular and colonial morphology, patterns of growth, nutritional requirements, technological properties and life cycle (see Chapter 4). Second, how brewing yeast is handled on a large scale. As with the fermentation process as practised in individual breweries, there is also a spectrum of sophistication in the manner with which the yeast is handled. In rare cases there is no need for yeast husbandry as such since fermentation results from spontaneous contamination of wort by the microflora of the fermenting hall. In this case the fermentation involves a mixture of several yeast types and bacteria. However, the majority of breweries use their own strains of yeast and these are jealously guarded. Historically these were selected from a wild population, by empirical means, on the basis of their possession of desired fermentation properties and ability to produce beer with a particular flavour. In many traditional breweries the same yeast may have been used continuously for very many years, inoculated into and recovered from successive fermentations and so on *ad infinitum*. In such cases the yeast is commonly a mixture of several strains, present in proportions which have been selected by the conditions of the particular fermentation and method of yeast handling.

In modern breweries it is also usual to inoculate a batch of wort with yeast derived from the crop of a previous fermentation. However, in order to ensure strain purity a newly propagated culture is periodically introduced (see Section 7.2). This does not preclude the use of mixtures of strains, although it is perhaps more difficult to control the relative proportions of each. In any case, in such modern breweries, for simplicity of handling there has been, for some years, a trend towards the use of single strains.

The practice of serial fermentation and periodic propagation has several consequences. It produces a requirement for recovering yeast from a fermenting vessel and then storing it under conditions that are both hygienic and capable of maintaining the yeast in a physiological condition appropriate for use in the next fermentation. Introduction of a new yeast culture into the brewery requires suitable plant in both the laboratory and brewery. This must be capable of producing a pure yeast culture in sufficient volume to achieve a desired inoculation rate in the first