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PROGRESS AND MODELLING OF COLD CONTACT FERMENTATION FOR ALCOHOL-FREE BEER PRODUCTION: A REVIEW

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ABSTRACT

Cold Contact Fermentation (CCF), or Cold Contact Process (CCP), is one of the many methods of producing beer with little to no alcohol content through a combination of low fermentation temperatures and extended fermentation contact times. Though this method was first discovered in 1983, its importance in academic and industrial circles has risen only recently, parallel to the rising demand for alcohol-free beer (AFB) recorded world-wide. For the discussion of this topic, the origins of AFB and the current market perspective of the sales and consumption of low or alcohol-free beer (L/AFB) serves as an introduction, followed by an exploration of the various methods of producing L/AFB. After these two introductory sections, an in-depth discussion of the biochemical pathways present in fermentation is presented as well as the mathematical basis upon which fermentation modeling stands in the form of differential and algebraic equation (DAE) modelling. Finally, a sequential review of the organoleptic properties of beer and the previously published fermentation system models in literature segues to the critical evaluation of this study. CCF, either with the use of free mass or immobilized yeast, is considered one of the best available production methods for producing AFB given the relatively minor additional capital investment and the ability to meet the various ethanol concentration specifications. However, several issues are discussed, most notably the difficulty reported in attenuating the contributions of negative flavor compounds that are generally reduced to higher degrees during standard fermentation practices.

1. Manufacturing and Global Perspectives

The nascent production of beer has ancient roots, developed in a multitude of cultures around the world as the result of agricultural surpluses in village societies.¹ It has grown from a localized artisanal or household activity to an industrial powerhouse of manufacturing and supply, with 1.95×10^{11} L produced globally in 2017.² As of 2014, beer was the second most consumed alcoholic beverage in the world, accounting for 34.8 % of all recorded alcohol consumption globally (Figure 1).¹

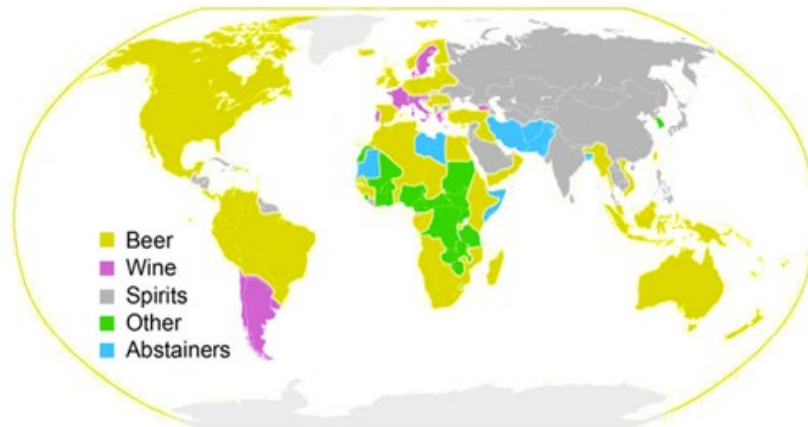
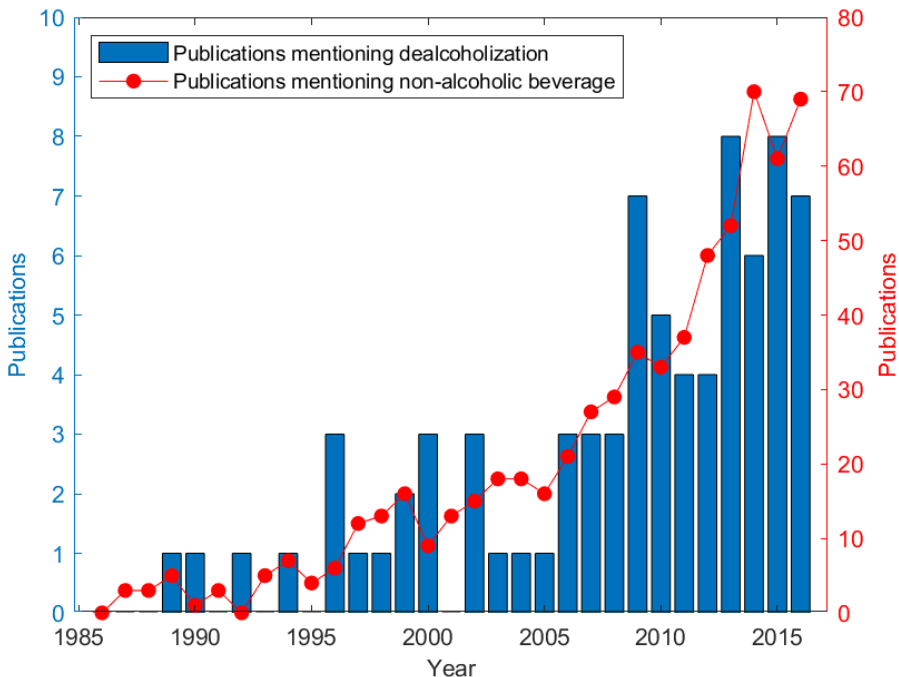


Figure 1: World Health Organization (WHO) 2014 records for beverage preferences worldwide.¹

34 There are several variations of beer, from Pilsners and Lagers (“bottom fermenting”) to Weissbiers and
 35 Ales (“top fermenting”).^{3,4} These can be further classified by alcohol strength (*i.e.* concentration) starting
 36 from alcohol-free beer (AFB) at 0-0.05% (v/v). The strength is defined by ‘alcohol by volume’ (ABV) in
 37 units of cm³ ethanol/100 cm³ beer or % (v/v).¹ The vast majority of beers reside in the range of 3-6% (v/v)
 38 though higher gravity brewing can produce beer with alcohol content up to 10% (v/v) or more, such as that
 39 produced in Trappist monasteries.¹

40
 41 **1.1 Alcohol Free Beer Manufacturing – An International Perspective**
 42 Though alcoholic beer is what one would expect to come to mind in Western countries when discussing the
 43 general topic of ‘beer’, the consumption of beer with low alcohol content or that is considered alcohol-free
 44 (L/AFB) is surging. Despite its recent debut as a consumer product, L/AFB saw an estimated increase in
 45 consumption of 80 % from 2007 to 2012, in the amount of 2.2×10⁹ L/year.^{5,6} Researchers have rationalized
 46 this trend as the junction of both increased legislative restrictions on consumption and the improved
 47 communication and awareness of the benefits of moderation.⁷ From a social perspective, alcohol
 48 consumption is linked with an increased risk of violent crime, traffic incidents and public disorder.^{7,8} With
 49 regard to the effects on the human body; ethanol is metabolized to acetaldehyde in the digestive system
 50 which binds cellular constituents and results in the creation of acetaldehyde adducts, which are damaging
 51 towards the body.⁷ Furthermore, efforts have been made to penetrate the markets in countries where alcohol
 52 consumption is forbidden under religious pretexts, leading to sales that would not have been garnered with
 53 the alcoholic version of beer.⁷ In addition to increases in consumption, the prevalence of L/AFB in
 54 industrial or academic research has also increased over the last three decades (Figure 2).⁹

55



56
 57 **Figure 2:** Graphical representation of the number of publications mentioning either the term
 58 “dealcoholization” or “non-alcoholic beverage” from 1986 to 2016.⁹ Re-synthesized from the original
 59 literature for clarity.

60
 61 This trend clearly shows L/AFB research to be gaining prominence as an increasingly significant topic of
 62 research. Historically, L/AFB production originated for a number of reasons. For example the shortage of
 63 raw materials in World War 1 and 2 led to beer production with reduced original extract (fermentable
 64 sugars), leading to a lower alcohol content.⁷ In addition, between the World Wars, alcohol production in

65 the United States of America was prohibited (1919 – 1933), incentivizing the production of AFB. ⁷ In order
 66 to pursue an in-depth review of L/AFB, it is necessary to first define what the terms “low” or “alcohol-free”
 67 mean quantitatively, as this provides a stringent constraint on the product in terms of both processing or the
 68 region of the world where it is sold. Counterintuitively, alcohol content specification for L/AFB varies
 69 greatly with the country where the sale is taking place (Table 1).
 70

Table 1: Compilation of mandated specifications for alcohol content for several countries in Europe and the United States. ⁵

Country	Low-alcohol beer (% v/v alcohol)	Alcohol-free beer (% v/v alcohol)
Denmark	–	< 0.10
United States	≤ 2.50	< 0.50
Portugal	≤ 1.20	< 0.50
Spain	≤ 3.00	< 1.00
United Kingdom	≤ 1.20	≤ 0.05
The Netherlands	≤ 1.20	≤ 0.10
Austria	≤ 1.90	≤ 0.50
Belgium	≤ 1.20	≤ 0.50
Finland	< 2.80	≤ 0.50
Germany	≤ 1.20	≤ 0.50
France	–	≤ 1.20
Italy	–	≤ 1.20
Sweden	≥ 2.25	–

71
 72 As a point of comparison with standards for countries that enforce religious prohibition, alcohol strength
 73 must not exceed 0.05 % (v/v) in some instances and must be completely absent in others. ^{5,7} Despite the
 74 ubiquitous focus on the damage associated with the consumption of alcoholic beverages, moderate beer
 75 drinking has been shown to be at least as effective as wine in reducing risks of coronary disease and heart
 76 attack. ⁷ In addition, beer provides some of the compounds and minerals part of a balanced and healthy diet
 77 such as polyphenols and magnesium (Table 2) as well as a fundamental lack of free sugars, fat and
 78 cholesterol that can be consumed through substitute beverages. ⁷
 79

Table 2: Table of the health related benefits of moderate beer consumption compiled from published literature. ¹⁰

Health Benefits	Bioactive beer constituents
Reduced risk of cardiovascular disease	Ethanol, phenolic compounds, B vitamins
Anticancer activities	Prenylflavonoids
Regulation of blood glucose levels	Beer
Improvement in lipoprotein metabolism	Ethanol
Stimulation of gastric acid secretion	Non-alcoholic components
Prevention of Alzheimer's disease	Beer
Lower risk of development of Parkinson's disease	Beer
Psychosomatic effects (eg. reduced stress)	Ethanol, hop compounds
Stimulation of cognitive function in old age	Ethanol
Sedative and hypnotic effect	Bitter hop compounds
Phytoestrogenic properties	Isoflavonoids
Antioxidant effects	Polyphenols, Maillard compounds
Isotonic drink	Beer
Source of minerals such as potassium and magnesium	Beer
Source of soluble fiber	Beer

80
 81 One may posit, therefore, that the consumption of L/AFB claims all of the benefits of beer consumption
 82 while both eliminating the social and physical damages and even providing a lower energy alternative (e.g.
 83 60.7% reduction in calorie content between a pale ale and a low-alcohol beer). ¹¹ Therefore, the impetus for

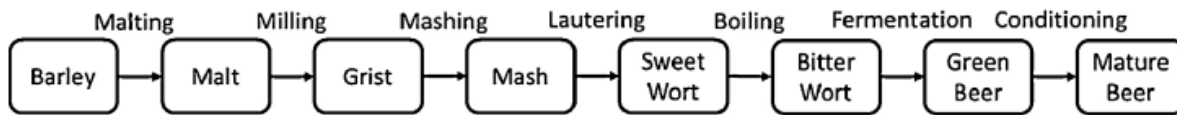
84 producing L/AFB is a function of cultural and societal changes but has beneficially led to the manufacturing
85 of a product with improved nutritional benefits over standard types of alcoholic beer.

86

87 1.2 Beer Manufacturing – The Malting and Brewing Processes

88 The core components of beer are water, barley malt, hops and yeast.¹² The beer manufacturing process
89 involves several steps (Figure 3). The process starts with using barley to create barley malt (“malting
90 process”) and leads to the brewing process which finishes with the conditioning steps.¹³

91



92 **Figure 3:** Block flow diagram of the different types of processes involved in the manufacturing of beer as
93 a consumer product.¹³

94

95 The malting process is performed first in order to simulate the grain’s natural germination cycle.¹¹ The
96 barley kernels are wetted and allowed to sprout, altering the starch filled interior.¹¹ This transformation
97 breaks down the hard endosperm into natural malt sugars that are then liquefied during the mashing process.
98 The malting process also produces the enzymes used in the mashing step. The kilning of malt then occurs
99 through the heating of malt to remove water before degermination and final storage prior to use during the
100 brewing process.¹⁴

101

102 The brewing process then commences at the milling stage.¹⁴ The malt mixture is milled, or broken down,
103 allowing for increase in the reactive surface area for enzymes and thus producing grist.¹⁴ The milling
104 process is an important step from a quality control standpoint. Metals and dust are removed at this step in
105 the attempt to avoid any equipment damage by friction and to prevent the occurrence of dust explosions
106 that could lead to serious injury or death.¹⁴ The husk is saved (generally) at this point to act as a filtration
107 layer during the lautering step.¹⁴ The grist is then added to water and this mixture is then mashed by forcing
108 stepwise increments in heating to activate carbohydrate and protein-degrading enzymes.¹¹ This process is
109 highly controlled, with constant monitoring of parameters such as pH, water-grist ratio (affusion) and
110 residence time.¹⁴

111

112 The mash then enters the lauter tun, with the aim of performing the separation of the liquids (wort) from
113 the solids (spent grist).¹⁴ This separation process produces the liquid ‘first wort’ at an extraction
114 composition of 16-20%.¹⁴ The remaining spent grist is then flushed with hot water, producing the ‘last
115 runnings’ of extract composition of 0.5-1%.¹⁴ This process is highly temperature dependent, with higher
116 temperatures resulting in improved lautering due to reduced processing viscosity but degradation of
117 enzymes critical to saccharification (such as α -amylase) above specific temperature thresholds (>80°C).¹⁴

118

119 The wort is then transferred to a kettle where it is boiled. At this stage, some brewers will incorporate
120 adjuncts such as corn syrup for sweetening depending on the specifications of the country of sale.¹⁴ Wort
121 boiling serves several purposes, the most important of which are the removal of dimethyl sulfide (DMS),
122 promoting the formation of flavor and color, enzyme degradation and flocculation. DMS is removed as it
123 is associated with cabbage or vegetable-like flavor and is not desirable in the wort mixture.¹⁴ Boiling
124 promotes evaporation and thus the removal of this compound. As it concerns flavor and color, this stage
125 produces the first instances of both melanoidin (antioxidant influencing color via Maillard pathway) and
126 Strecker aldehyde formation.^{12,14} The flocculation component refers to the conglomeration of proteins,
127 attributing to positive attributes such as foam and taste in mature beer. Hops are also added at the wort
128 boiling stage. This is accomplished either at the beginning or the end of boiling depending on the brewer’s
129 preferences and the type of flavor desired. The addition of hops serve to add bitterness and flavor while

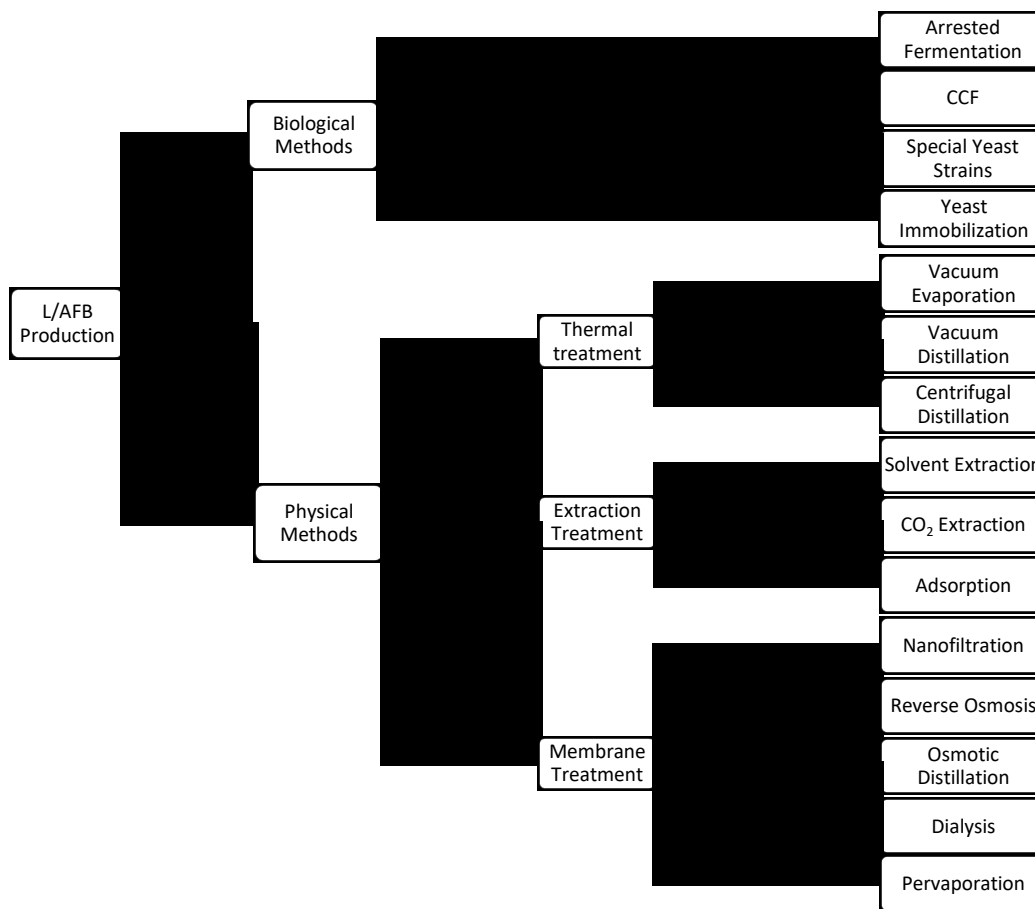
130 enhancing foam formation and stability.¹² ‘Hot trub’ – the hop and precipitated proteins – are then removed
131 in a whirlpool after boiling in order to prevent the impeding of yeast activities downstream.¹⁴ The remaining
132 wort is then cooled and aerated (5-10 °C for bottom fermentation and 15-25 °C for top fermentation).¹⁴ In
133 the case of Cold Contact Fermentation (CCF), cooling to within 0-1 °C is typical prior to pitching (mixing
134 cooled wort with yeast) in the fermenter.⁵

135
136 At the fermentation stage, the cooled wort is mixed with yeast and a small amount of air to promote the
137 growth of yeast.¹² This is done quickly to prevent the development of bacteria.¹⁴ The goal of fermentation
138 stage is to promote the consumption of fermentable sugars by the yeast, resulting in the ‘final attenuation’,
139 signaling the completion of the fermentation phase based on fermentable sugar concentration.¹⁴ Here, as
140 with lautering, temperature is of primary importance. The temperature influences the multitude of rates of
141 reaction occurring over the course of the fermentation period and by extension the formation of any
142 secondary flavor products. For the various cases for producing alcoholic beer, temperatures can range from
143 6-22°C for a total contact period of 5-21 days. However, literature sources indicate the CCF method makes
144 use of a combination of fermentation contact times of 24-100 hr with reduced temperatures of 0-8°C so as
145 to inhibit the formation of ethanol while maintaining the yeasts’ metabolism of secondary flavor substrates.
146 ^{5,15-18}

147
148 The fermentation step produces ‘green beer’ with residual extract of 6-10% which contribute to CO₂
149 formation in maturation.¹⁴ It is important to note that the final beer product should be absent of residual
150 extract as this serves to reduce digestibility and increases the risk of infection.¹⁴ The final product is then
151 ‘washed’ by CO₂ bubbling to remove aldehydes and provide additional carbonation before being stored at
152 ~0°C, though the method of storage is product dependent and should not be overly generalized.¹⁹ For the
153 final stabilization (rounding of off-color and improving the flavor) and clarification, a number of tasks are
154 performed. These are Kieselguhr filtration, the addition of stabilizing agents, product conservation and most
155 importantly the natural maturation that is promoted in the container where ageing occurs.¹⁴

156
157 A number of different approaches depend on the brewer’s preferences. For instance, the gravity of the
158 mixture after wort boiling for most beer is typically between 11–12%.¹⁴ However, high-gravity brewers
159 alter the gravity of the wort at this stage to ~16–20 wt%. Types of heating can vary substantially as well for
160 the fermentation step, ranging from base heating to external boiling. At the boiling stage, boiling can also
161 take place at or below atmospheric pressure depending on whether the acceleration of physical processes
162 or volatile separation is desired. Pressure can also be applied to reduce yeast propagation and thus reduce
163 the reaction rate.¹⁴ Typical yeast dosage is on the order of $1.5 \times 10^7 - 3 \times 10^7$ cells mL⁻¹, depending on the
164 desired gravity with higher gravity methods requiring more yeast.¹⁴ Mixing, either through natural
165 convection and/or stirring aids in increasing the heat transfer in the vessel and preventing hot-spots and is
166 also a contentious subject between modern and traditional brewers.

167
168 **2. Methods of Dealcoholization**
169 CCF, also known as cold contact process (CCP), is one of the methods utilized for the inhibition of alcohol
170 formation and was first proposed by Schur in 1983.^{15,18,20} Despite its primary importance for this review,
171 an extended review of all the methods available provides hierarchical context for categorization and
172 improved understanding of processing differences. To this end, several alternative methods are detailed
173 below that either seek to inhibit alcohol formation during fermentation or remove it through post-
174 fermentation processing (Figure 5).⁵



175
176 **Figure 5:** Flow chart of the different types of processes available for producing L/AFB. ^{5,21} Re-synthesized
177 from literature.

178
179 When comparing the industrial execution of these methods, the adjustment of the brewing process in order
180 to limit the production of ethanol is considered the most common. ⁵ Dealcoholization methods can be
181 organized into either “biological” or “physical” categories, whereby biological sub-categories employ pre-
182 processing methods and physical sub-categories employ post-processing methods. Figure 5 shows a
183 generalized view of the state-of-the art, with some allowances for specific nomenclature. For instance,
184 “Special Yeast Strains” can refer to either the use of genetically modified yeast strains or the use of atypical
185 strains such as *Saccharomyces ludwigii* in lieu of *Saccharomyces cerevisiae* (Brewer’s Yeast). ⁵ The
186 nomenclature of some terms have been adjusted for clarity from what is presented in literature, as well. For
187 instance “Adsorption” can refer to the separate use of different media such as zeolites, resins or Kieselgels,
188 though combined here into one category. ^{5,21} In addition, “Centrifugal Extraction” has been referred to
189 analogously as “Spinning Cone Distillation” in some instances. ^{5,21} The term “Vacuum Evaporation”
190 includes falling film evaporation. Also, “Yeast Immobilization” implies includes the use of immobilized
191 yeast in concert with the CCF methodology. Finally, it is important to note that a combination of the
192 methods shown in Figure 5 can be employed to achieve specified outcomes. ^{5,16}

193
194 **2.1 Overview of Pre-Processing Methods**

195 A comparison of the advantages and disadvantages of each method has been developed as an overview
196 (Table 3). A more in-depth discussion of pre-processing methods can be found in section 2.3. The use of
197 CCF or special yeast strains appears the most advantageous by virtue of the ratio of disadvantages to
198 advantages in comparison to other methods. However, the provision of an absolute conclusion is premature

199 without greater analysis of the quantitative implications of different factors on beer quality, as the relative
 200 significance of any one advantage or disadvantage is absent from literature.
 201

Table 3: Table of the advantages and disadvantages of pre-processing methods for the inhibition of ethanol formation. ²¹

Process	Advantages	Disadvantages
Arrested Fermentation	-Uses the standard fermentation equipment	-Restricts the formation of aroma compounds -Worty aroma
CCF	-Uses the standard fermentation equipment -Reduced carbonyl compounds -Produces aroma compounds -Achieves ethanol content of 0.05 % v/v	-Conversion of amino acids to aldehydes -Incomplete conversion of Strecker aldehydes
Special Yeast Strains	-Uses the standard fermentation equipment -Achieves ethanol content of 0.05 % v/v	-High sugar content (sweetness) of final product
Yeast Immobilization	-Reduced aldehydes by yeast consumption -Formation of new aroma compounds by yeast -Improved utilization of raw materials	-Difficult to control -High carrier price -Contamination risks -Continuous bioreactor needed

202
 203 **2.2 Overview of Post-Processing Methods**
 204 Post-processing methods for the removal of ethanol have been compiled and compared in light of their
 205 respective advantages and disadvantages from a high level perspective (Table 4). The number of post-
 206 processing methods available for the removal of ethanol after fermentation are approximately three fold
 207 greater than for pre-processing. In addition, a comparatively large amount of literature is available for post-
 208 processing methods. Overall, the economic feasibility of post-processing methods is impeded due to the
 209 requirement for additional equipment in excess of the standard brewery unit operations as well as the energy
 210 intensiveness of some unit operations (*i.e.* distillation). These factors offset profits for existing plants and
 211 retard return on investment (ROI) for new ventures.
 212

213 **2.3 A Discussion of Pre-Processing Methods**
 214 Post-processing methods for manufacturing L/AFB have potential for success. This is partly due to the
 215 control available in selectively reincorporating aroma compounds (measured *in situ*) after processing that
 216 are either separated or degraded due to thermal contact. ⁹ In addition, there are difficulties that arise with
 217 pre-processing methods that revolve around process control issues, typically due to the altered production
 218 rates of secondary flavor compounds or the incomplete consumption of sugars. ^{9,21-23} However, in the
 219 interest of maintaining a sufficiently refined scope, discussions of the processing conditions involved for
 220 post-processing methods (Table 4) have been omitted. Instead, pre-processing methods will be discussed
 221 further, given their relevance and method similarity for L/AFB production.
 222

223 Varying efficacies are encountered when employing either of the four pre-processing methods described
 224 previously with respect to the inhibition of the formation of alcohol (Table 5). The composition of ethanol
 225 in the final product can be very similar between methods, with varying difficulties when using any method.
 226 ^{14,16,21,24,25} Typically, when applying any of these biological methods, worts with a low concentration of
 227 fermentable carbohydrates are used (e.g. 25–30% for L/AFB in comparison to 80% for pale ales) given the
 228 anticipated incomplete consumption of sugars. ⁵ The concentration of fermentable carbohydrates is altered
 229 in the mashing phase, whereby the decoction is removed, boiled and then reintroduced to the wort mixture.⁵
 230

231 Arrested fermentation in particular is characterized by a high sulphur content, allowing for DMS to be used
 232 as an analytical marker. ⁵ Studies based on arrested fermentation with the use of a packed bed reactor have
 233 been successful (though described dubiously as “optimal”) even while operating within the CCF
 234 temperature range, as a result of higher control and lower contact times with respect to the free mass yeast
 235 method. ²⁶
 236
 237

Table 4: Table of the advantages and disadvantages of post-processing methods for the removal of ethanol. ²¹

Process	Advantages	Disadvantages
Vacuum Evaporation	-Achieves ethanol content of 0.05 % v/v -Moderate temperatures needed	-Requires evaporator -High energy costs -Thermal impact to heat sensitive compounds -Co-distillation of aroma compounds
Vacuum Distillation	-Achieves ethanol content of 0.05 % v/v -Moderate temperatures needed	-Requires distillation column -High energy costs -Thermal impact to heat sensitive compounds -Co-distillation of aroma compounds
Centrifugal Distillation	-Achieves ethanol content of 0.05 % v/v -Minimal thermal impact -Low residence time	-Requires spinning cone column -High energy costs -Removal of volatile compounds with -stripping medium
Solvent Extraction	-Solvents immiscible with water yet highly soluble in ethanol	-Requires liquid-liquid extraction unit -Aroma compounds removed in solvent -Trace remains of solvent in product -Solvents must be compliant with food standards
Carbon Dioxide Extraction	-Selective removal of ethanol without removing water/larger aroma compounds -Room temperature application	-Requires additional equipment -Carbon dioxide strips volatile compounds -High operation costs
Adsorption	-Adsorbents have good affinity with ethanol	-Additional unit required -Adsorbent regeneration required -Co-adsorption of aroma compounds with ethanol -High operation costs
Nanofiltration	-Low temperature and pressure -High retention to aroma compounds	-Requires nanofiltration unit -Requires diafiltration water
Reverse Osmosis	-Low pressure and temperature -Some high retention towards aroma compounds	-Requires membrane unit -High pressure non-ideal with beer -Some low retention to aroma compounds -Requires diafiltration water -Difficulty achieving <0.45% v/v ethanol
Osmotic Distillation	-Low temperatures -Water permeation is reduced	-Requires additional separation unit -Requires recirculation of stripped solution -Loss of aroma compounds
Dialysis	-Low temperatures -No water permeation	-Requires dialysis unit -Requires dialysate recirculation -Loss of aroma compounds
Pervaporation	-Low temperatures -Increased ethanol removal with hydrophilic membranes -Reduced water extraction through use of sweep gas with steam	-Requires pervaporation unit -Hydrophobic membranes promote higher aroma compound removal -Requires high membrane areas due to low permeation flux -High costs of vacuum and condensation

238
239 Though the category of “Special Yeast Strains” (Table 5) can meet the L/AFB requirement of 0.05% (v/v),
240 yeast strains such as *Saccharomyces ludwigii* do not consume maltose, resulting in a very significant
241 flavor profile detriment of excessive sweetness. ⁵ The genetic modification of Brewer’s Yeast to be ‘Alcohol
242 dehydrogenase-free/negative’ have produced positive results with regard to inhibiting ethanol production
243 but result in the accumulation of acetaldehyde and, once again, excessive sweetness. ¹⁶ Though theoretically
244 the best option if perfected as genes govern cell functions, the use of genetically modified yeasts has resulted
245 in elevated levels of acetaldehyde, diacetyl and acetoin. This produced a beverage more similar to sherry
246 than beer. ²⁵
247

248 CCF has been documented to produce ethanol concentrations similar to the other methods as seen in Table
249 5 but was recorded lowest (0.02% (v/v)) in the original work by Schur. ¹⁸ CCF requires a higher yeast/cell
250 ratio on the order of 30×10^6 10^8 cells mL⁻¹ and increased energy intensiveness in the form of cooling to
251 within 0°C, as stated previously. ^{5,23} With CCF using free mass yeast, wort can be stripped at low
252 temperature (0°C) and under pressure with carbon dioxide, helping to eliminate the sulphur compounds

253 normally removed during standard fermentation. A contact time of 24–100 hours is then used. Combined
 254 with super high gravity (SHG) processing (18°P), beer with less than 0.1% (v/v) can be produced. ⁵ The
 255 SHG brewing serves to increase the ester and alcohol formation at later steps. ⁷ CCF processes performed
 256 in a laboratory environment have shown the need for chemical acidification, as the pH in batch is higher
 257 than what is typically reported for standard fermentation. ^{5,15} In addition, elevated levels of several flavor
 258 compounds has been noted, including methional and some Strecker aldehydes. ¹⁵
 259

Table 5: Table detailing some of the typical values and ranges for ethanol % (v/v) using different pre-processing methods. ^{5,17,18}

Pre-processing Method	% (v/v) ethanol
Arrested Fermentation	0.3–1.0
CCF	0.02–0.64
Special Yeast Strains	0.05
Yeast Immobilization	0.22–0.42

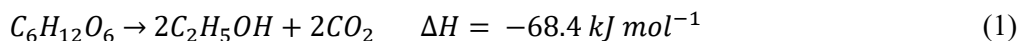
260
 261 CCF with immobilized yeast requires less time and has improved yeast reuse potential but is even more
 262 difficult to control than the standard CCF method and requires a continuous bioreactor. ⁵ Despite these
 263 drawbacks, the use of immobilized yeast technology for producing L/AFB has been described as the “most
 264 successful”. ¹⁷ The different immobilization techniques can be divided as follows: surface attachment to a
 265 solid support of metal oxides/amilosilanes, entrapment inside a porous matrix such as synthetic polymeric
 266 hydrogels, containment within a barrier such as microcapsules and self-aggregation through natural
 267 flocculation. ⁵ Laboratory results have even shown a 70% drop in Strecker aldehyde concentrations using
 268 immobilized yeast techniques with CCP as well as a three to five-fold increase in NADP-specific activity
 269 (towards the reduction of branched chain aldehydes) compared to free mass anaerobic cells. ^{15,27}
 270

271 3. Yeast and Biochemical Pathways

272 The yeast strains present in the brewing process are fundamental to the flavor and aroma profile produced
 273 in beer, of which it is estimated there are 200+ key species. ²⁸ The biochemical pathways present through
 274 either the metabolic (occurring inside of the cell) or non-metabolic pathways produce a myriad of flavor
 275 active compounds through an enormous number of chemical pathways. These have been condensed here to
 276 represent the routes most critical to ester, aldehyde, ethanol and higher (fusel) alcohol synthesis.
 277

278 The most important genus for producing L/AFB successfully other than *Saccharomyces* is
 279 *Saccharomyces ludwigii*. ^{23,25} The hybrid strain *Saccharomyces pastorianus* (formerly *Saccharomyces*
 280 *carlsbergensis*) and *Candida Shehatae* have also been used in industrial and academic environments for
 281 producing L/AFB but are less common. ^{25,29} Given the low temperature range typical of CCF as stated
 282 previously, strains used during the production of lager beers appear to be the most sensible choice for CCF
 283 outside of those noted above given the overlap in acceptable operational temperatures at 7–8°C. ²⁹
 284

285 From a brewer’s perspective, the most important reaction occurring during fermentation is the conversion
 286 of wort sugars to ethanol and carbon dioxide, as represented by the Gay-Lussac equation,
 287

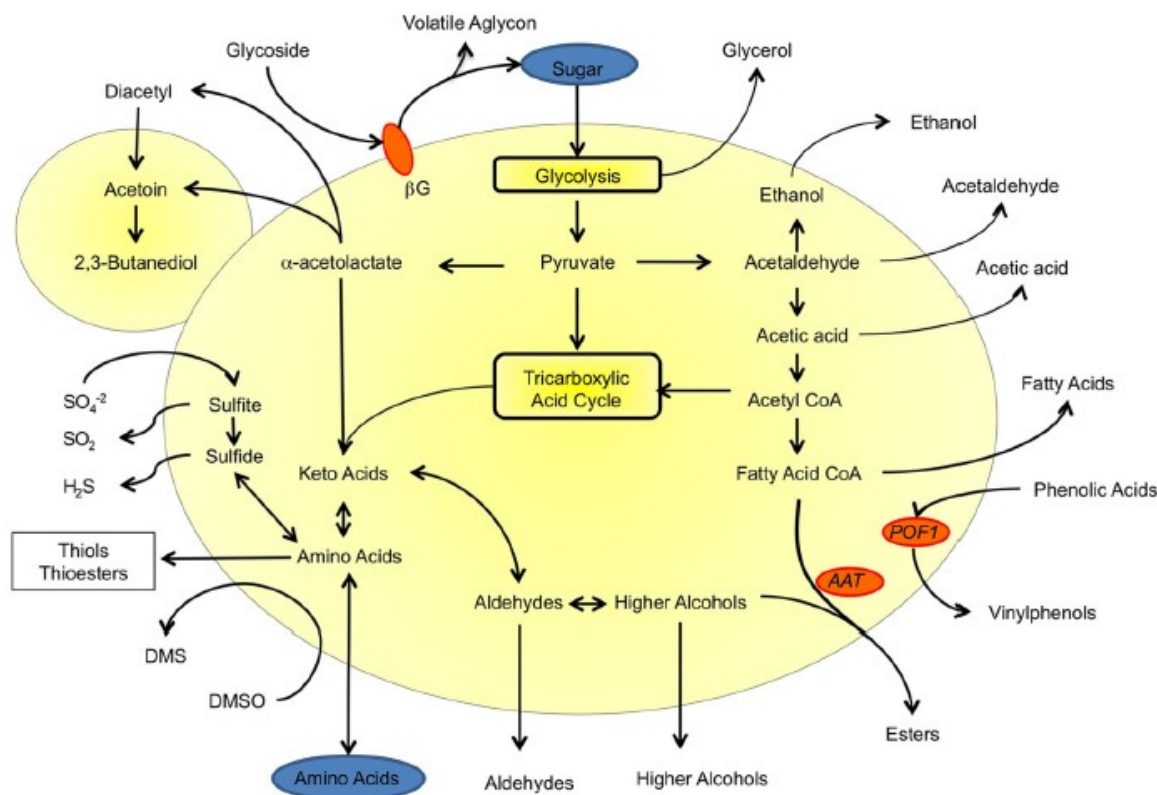


289 However, this equation details the beginning and end of fermentation with no mention of the complex
 290 pathways occurring in-between. ²¹ As such, the bulk of this section is centered on the disambiguation of
 291 those complex pathways that both allow ethanol to form and are complementary to its synthesis.
 292

293 Generally speaking, all carbonyls are formed in beer through three main reaction pathways: Maillard
 294 reactions between amino acids and sugars, Strecker aldehyde degradation of amino acids and lipid
 295 degradation (including oxidation, autoxidation, photo-oxidation and enzymatic oxidation). ²⁷ Interestingly,

296 Strecker aldehyde and lipid degradation are also intermediates of metabolic pathways of yeast. ²⁷ Other
 297 pathways that produce carbonyls include imine formation pathways, melanoidin-catalyzed oxidation of
 298 higher alcohols, aldol condensation, Amadori compound degradation and the degradation of bitter acids. ³⁰
 299 However, these other pathways are omitted here for brevity.

300
 301 Fusel alcohols result either from catabolic assimilation of amino acids or the anabolic metabolism of sugars
 302 in the cell. ²¹ Fusel alcohols are crucial as they are a precursor of esters, which can be classified as either
 303 acetate or ethyl esters. ²¹ Acetate esters are produced in higher quantities and have been much more heavily
 304 documented. ³¹ The biochemical pathways for the production of these key compounds are described at a
 305 high level in Figure 6.
 306



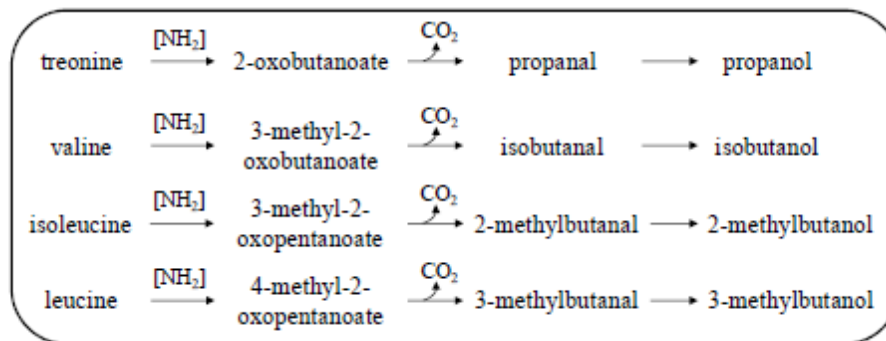
307
 308 **Figure 6:** A pictorial representation of the metabolic activities of *Saccharomyces* strains that influence beer
 309 flavor and quality. ³²

310
 311 In general; lipids, fermentable sugars, diacetyl, amino acids, oxygen and nitrogen are used by yeast in order
 312 to produce the alcohols, esters, acids, sulphur compounds and aldehydes experienced during the tasting of
 313 beer. ^{21,31,32} Overall, the effect of yeast during fermentation is extremely complex. ³³

314
 315 **3.1 Metabolic Pathways**

316 Fusel alcohol synthesis occurs using oxo-acids (Figure 7). The difference between catabolic and anabolic
 317 routes are the source of oxo-acids, with the catabolic route requiring that the wort amino acids are
 318 assimilated by the yeast whereas through the anabolic route the oxo-acids are generated from pyruvate
 319 within the cell. ²¹ In either case, the oxo-acid is decarboxylated to an aldehyde and then reduced to the
 320 corresponding alcohol. ²⁴ The only caveat lies with *n*-propanol, which is only produced via anabolic routes
 321 given the absence of a corresponding amino acid. ²⁴

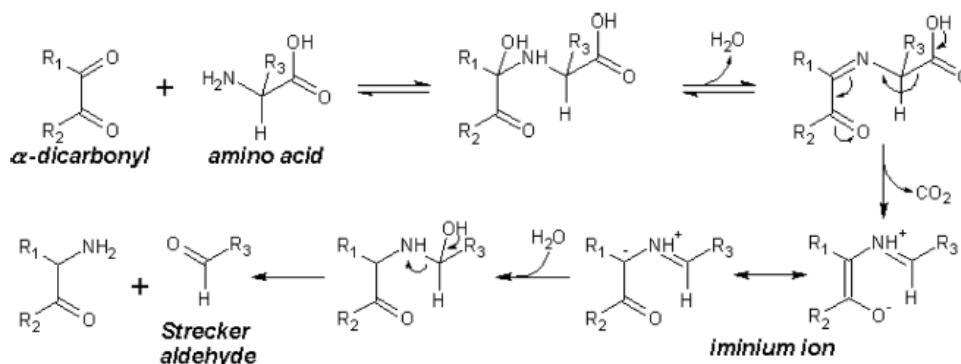
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Figure 7: Outline of the formation of fusel alcohols by amino acid metabolism.²¹

Esters are the most important positive flavor-active compounds in beer despite only being present in trace amounts.^{24,34} Acetate esters are synthesized by the transesterification of acetyl-coenzyme A (acetyl-Co-A) and since acetyl-Co-A is an intermediate in the biosynthesis of lipids, ester production is tightly linked to lipid metabolism in yeast (Figure 6).²⁶ Ester production also occurs through enzymatic condensation reactions of organic acids and alcohols.³⁴ Ethyl acetate is the most common ester present in beer as it is directly linked to the formation or existence of ethanol, which is still present with standard beer or L/AFB processing.²¹ Transamination can occur between an amino acid and an α -dicarbonyl, resulting in the “Strecker degradation” seen below (Figure 8).



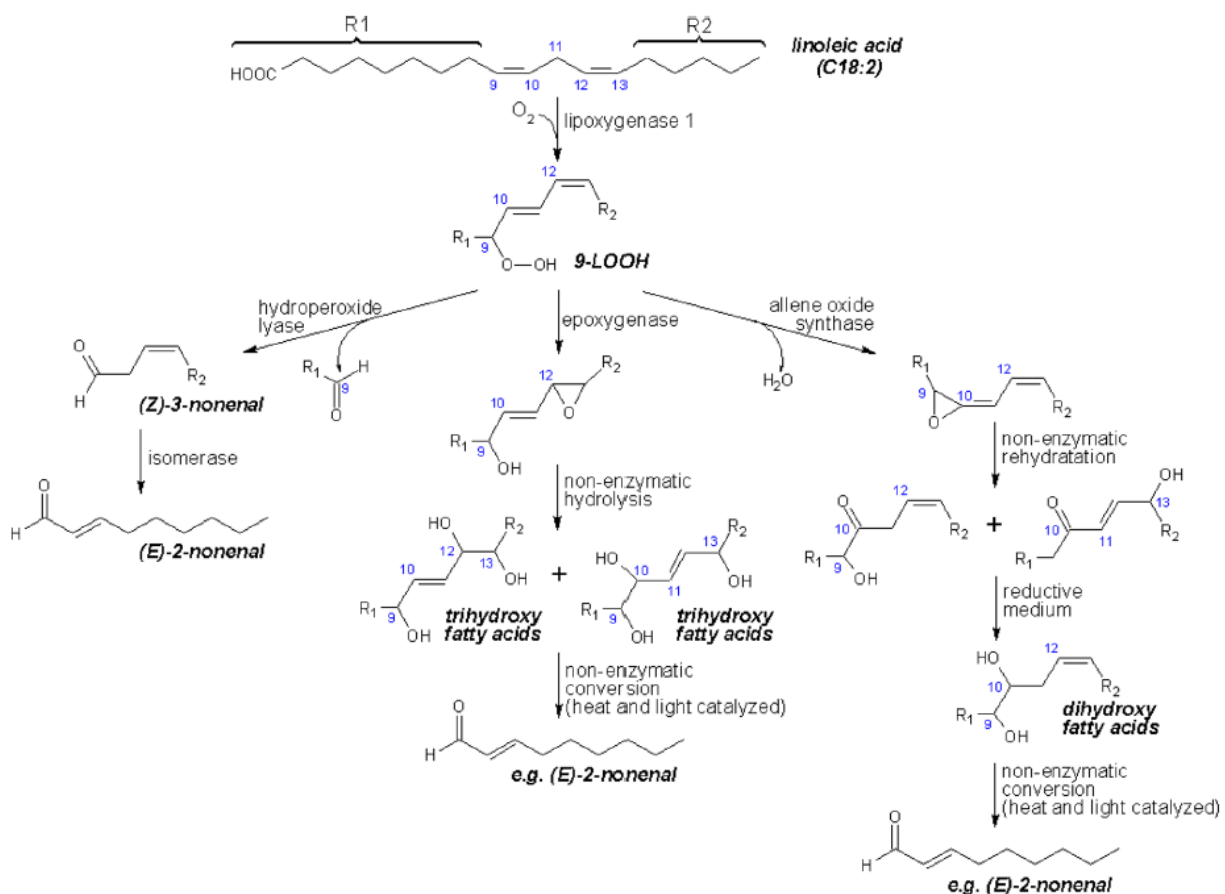
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Figure 8: The Strecker degradation for an α -dicarbonyl reacting with an amino acid.³⁰

The other metabolic pathway of great interest is lipid degradation. Enzymatic oxidation of lipids is shown (Figure 9). The (Z,Z)-1,4-pentadiene structure in linoleic and linolenic acid is key to the oxidation pathway, resulting in hydroperoxy acids which are then converted to fatty compounds and then carbonyls.³⁰

3.2 Non-Metabolic Reactions

One of the non-metabolic reactions present are referred to as Maillard reactions (Figure 10).³⁰ These occur at 50 °C within the range of pH 4–7 and are responsible for the formation of color in beer, as stated previously.³⁰ Maillard reactions generate a vast and diverse set of products. However, furfural is of particular interest from a quantitative perspective and are used as indicators of the heat load placed on the beer (through any stage in either mashing or brewing process) as well as for general flavor staling as their concentrations increase linearly throughout brewing.³⁰ Researchers have contradictory views on the overall impact of furfural towards beer taste, despite the agreement that Maillard reactions continue during maturation.³⁰ As a final note, it is important to recognize that the Strecker degradation pathways and the Maillard reactions are interconnected given the formation of α -dicarbonyls formed in Figure 10, so a strict delineation between metabolic and non-metabolic reactions, void of connections, is unrealistic.



355
 356 **Figure 9:** The enzymatic breakdown of linoleic acid based on some of the relevant published pathways.³⁰
 357 The epoxygenase and allene oxide synthase paths produce an enormous number of potential aldehydes and
 358 ketones, such as trans-2-nonenal.³⁰

359

360 4. Organoleptic Properties

361 Given that beer is not only a consumer beverage but an important part of many cultures and traditions, it is
 362 evident that ensuring positive and consistent flavor profiles in beer is paramount for product success and
 363 longevity. However, as seen in section 2.1, the main drawbacks of making use of biological methods such
 364 as CCF to produce L/AFB are related to deficiencies in flavor and aroma such as sweetness, worty off-
 365 flavors, absence of positive aromas and bitterness.^{21,35} Because of this, the key process indicators (KPIs)
 366 for CCF are indicators of positive flavor profiles, all while adhering to the appropriate standards for ethanol
 367 content and continued recognition of the standard KPIs of pH and residual extract that are typical of brewing
 368 in general. However, to complicate matters, given the vast number of compounds present in beer, it is very
 369 possible for a beer to have both the physical and chemical properties within accepted levels and yet be
 370 unacceptable in taste.³⁶ The understanding of flavor and its development in a mixture as complex as beer is
 371 a fundamental step towards ensuring processing consistency, flexibility to change and potential for
 372 improvement.

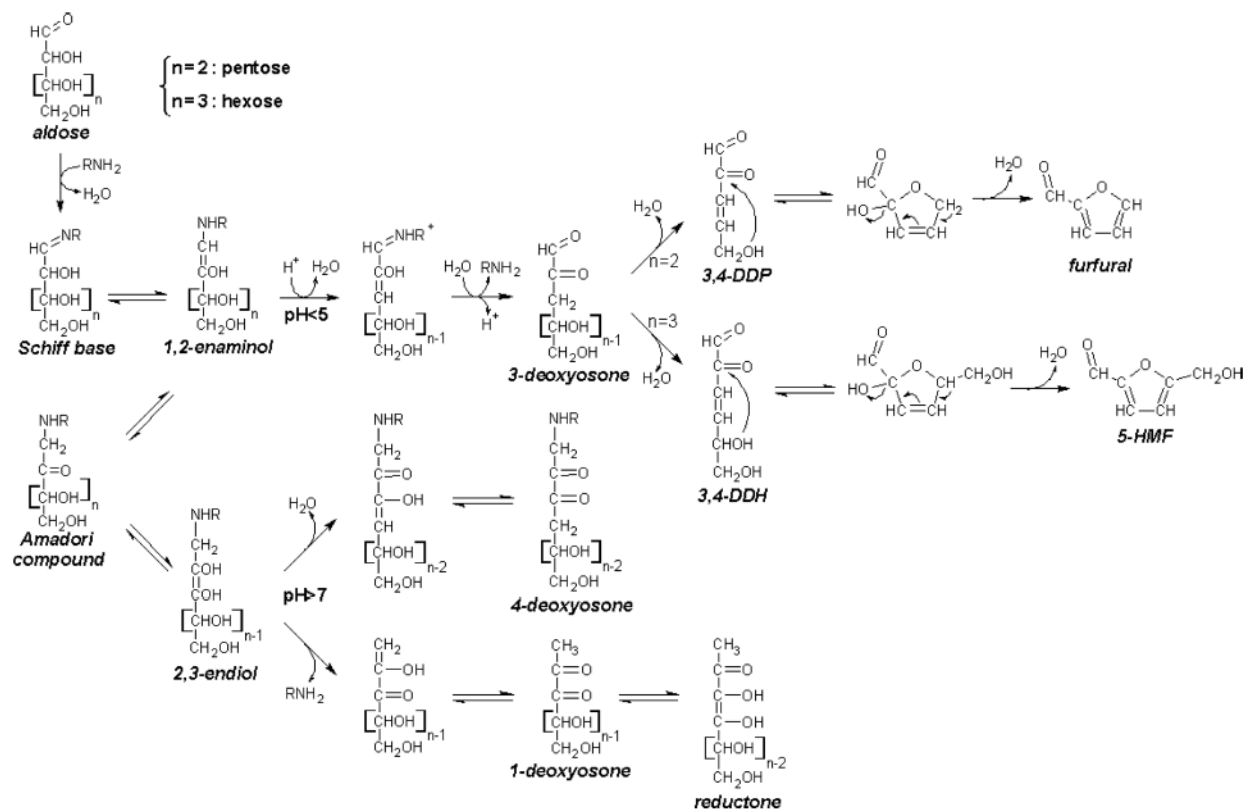
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374 Flavor is defined as the sum of perceptions resulting from stimulation of the sense ends that are grouped
 375 together at the entrance of the alimentary and respiratory tracts. Flavor is said to be comprised of four
 376 different components namely; odor, aroma, taste and mouthfeel.³⁰ Odor refers specifically to the perception
 377 of volatiles by the olfactory membrane in the nasal cavity whereas aroma is the sensation of the
 378 volatilization of compounds in the mouth due to natural body heat, thus reaching the nasal cavity in a

379 retronasal fashion.³⁰ Taste refers to the perception of soluble substances on the tongue, and in turn to any
 380 of the six taste attributes (sweet, salty, sour, bitter, umami, fatty). Mouthfeel refers to the haptic perception
 381 on the oral cavity surface, e.g. the alcohol warming effect or the carbon dioxide bubbling sensation.³⁰

382
 383 Despite these rigorous categorizations, flavor is a composite perception with all elements inter-connected.
 384 In addition, the presence of a certain compound may act to increase or diminish the perception of another
 385 compound, a phenomenon aptly named “synergy” or “suppression”, respectively.^{23,30} This complicates the
 386 definitions, as it has been shown that two or three aldehydes in a mixture, each at their individual
 387 subthreshold level, have had a perceivable effect on flavor.³⁰ One of the best known tools for sensory beer
 388 flavor detection is the beer flavor wheel: this tool was created in an effort towards comprehensive
 389 standardization of the terms used to describe the sensory characteristics of beer.³⁷ Several newer models
 390 that build on this first effort towards standardization have been developed since for improvement.^{30,36-37}

391



392
 393 **Figure 10:** The Maillard reaction pathways for pentose (n=2) and hexose (n=3), resulting in α-dicarbonyls
 394 (deoxyosones) and heterocyclic compounds such as furfural and 5-HMF.³⁰

395

396 4.1 Processing Factors Affecting Flavor

397 During the brewing process, several factors must be considered in order to produce a beer of sufficient
 398 flavor quality and character. The quality and type of ingredients such as barley, water or hops have a large
 399 impact on flavor.³⁸ The other method of influencing flavor is the manipulation of processing conditions
 400 during brewing and therefore directly affecting the metabolism of yeast. Here, the process manipulations
 401 (after the mashing stage) that affect flavor are subdivided into either pre or post-bottling categories.

402

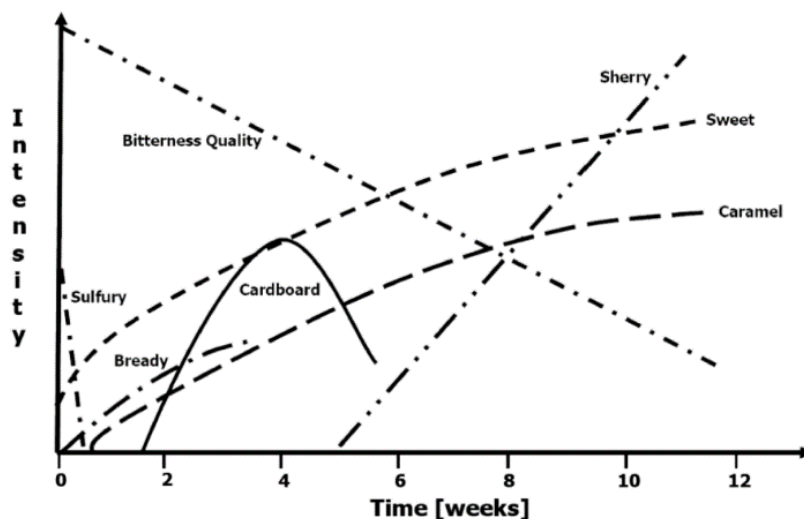
403 One of these core processing factors affecting flavor is the health and amount of yeast being pitched. The
 404 quality of the yeast is referred to in terms of the “viability” and “vitality”.³⁰ Viability refers to the cells’
 405 ability to grow, reproduce and interact with their environment whereas vitality is seen as a measure of
 406 activity, fermentation performance or the ability to overcome physiological stresses. Yeast quality is

407 influenced by factors such as wort clarity, wort oxygenation, pitching-rate, temperature and lipid
408 composition.³⁰ However, these external factors do not always provide the same result when comparing
409 between yeast strains and processing methods.²³ For instance the hypothesized effects of increased
410 temperature and pitching-rate with the use of genetic mutants of *Saccharomyces pastorianus* during arrested
411 fermentation were not achieved in some cases, namely the improvement of flavor compound production.²³
412 This leads to researchers adding flavor compounds after fermentation to mask warty off-flavors with potent
413 compounds such as isoamyl alcohol and isoamyl acetate.^{21,23}

414
415 In addition, wort boiling influences final product flavor, aiming to precipitate unwanted nitrogenous
416 substances, stop enzymatic processes, sterilize the batch and volatilize excess hop-oil.³⁹ Worts boiled for
417 longer periods of time, however, provide more bitter flavor profiles but also higher stability and less
418 retention of head. The combination of higher mashing temperatures and longer boiling served to increase
419 flavor stability and shelf life, though with diminishing returns for mash temperatures at or above 63°C.³⁹

420
421 Most importantly, fermentation influences beer flavor and quality tremendously, most notably with flavor
422 stability. The control of pH in particular during fermentation appears to be yeast strain dependent and a
423 large part of flavor stability.³³ The main influencing factors on yeast metabolism are temperature and batch
424 contact time, particularly with regards to the production of aldehydes.⁴⁰ As such, these factors are of primary
425 concern during CCF. The type of reactor, as well as the fluid dynamics in reactors have also been studied
426 with respect to flavor production, showing that changes in conditions such as hydrodynamics, reactor
427 geometry and shear stress can provide a roughly fivefold reduction in ethanol content with genetically
428 modified yeast (roughly threefold for non-modified strain) while also providing an increase of positive
429 flavor compounds for batch geometries.¹⁷ Despite manipulation variables available during manufacturing,
430 post-bottling maturation occurs on the shelf, resulting in flavor developments beyond the immediate control
431 of the brewery (Figure 11).

432



433
434 **Figure 11:** Representation of the changes in different flavor intensities with maturation time post-bottling.³⁰
435

436 This is not to be confused with the variations in maturation of green beer that occurs in varying
437 circumstances such as in cellars, caves, vats, barrels and casks which also contribute to the flavor profile.
438 ¹⁹ This post-bottling maturation can be associated with both negative and positive changes in flavor. To
439 further complicate matters, a staling effect need not be just associated with an increase in negative flavor
440 contributors but just a decrease in positive flavor contributors.³⁰ For instance, aldehydes formed during
441 production have shown to be chemically bound to other compounds, obscuring them from sensory detection
442 until post-maturation. However, given the lack of chemical equilibrium present in the bottle, the bound

443 aldehydes are hypothesized to become unbound, causing staleness in flavor. The excessive changes to
444 flavor during post-bottling maturation infer a lack of sufficient organoleptic stability.³⁰
445

446 Of all the possible causes of accelerated staling, increases in temperature are the most significant root cause
447 and unsaturated aldehydes such as *trans*-2-nonenal considered most to blame with respect to cardboard-
448 like flavors.^{12,30,36} As a rule of thumb originating from the Arrhenius expression, a 10°C increase in
449 temperature roughly doubles the rate of chemical reactions.³⁰ However, flavor loss during storage are not
450 the only concern as the healthful effects of many antioxidants are removed during ageing.³⁶
451

452 **4.2 Negative Flavor Contributions**

453 The effects of negative flavor compound concentrations can render beer unpalatable. These can be
454 categorized as flavor profiles developed during the brewing process (post-mashing through ageing) or
455 during the post-bottling maturation process, as discussed in section 4.1. It is no secret that L/AFBs differ
456 fundamentally from normal beer in terms of flavor. Unmentioned up to this point is the influence of lower
457 ethanol content which is integral to the water flavor compound matrix of the final product, resulting in less
458 retention of some positive flavor active compounds but greater perception of wort-flavored aldehydes.^{9,23,41}
459 However, given that alcohol content is subject to strict specification constraints, the understanding and
460 manipulation of the production of secondary flavor compounds becomes even more critical to ensuring the
461 integrity of brews, especially given their presence as the main disadvantage of CCF and other biological
462 methods.
463

464 Vicinal diketone (VDK) content is of primary importance when discussing the negative flavor profiles of
465 beer as they are used to differentiate green from aged beer and are somewhat associated with worty off-
466 flavors.³⁸ These are compounds such as diacetyl (2,3-butanedione) and 2,3-pentanedione, which are
467 produced as by-products of amino acid metabolism.^{12,25,38,42} During processes such as CCF, wort aldehydes
468 are reduced by the alcohol dehydrogenase activity of yeast, leading to a balance of ethanol and VDKs.²⁶
469 More generally, aldehydes can pose serious risk to a palatable flavor profile in beer and as seen before are
470 produced by the oxidation of fatty compounds and alcohols. They can be reduced to ethanol by the end of
471 primary fermentation, though the presence of oxygen will reverse this process.³⁸ The aldehydes (other than
472 VDKs) that influence beer flavor the most are 2-methylbutanal, furfural, isobutyraldehyde, acetaldehyde,
473 2-phenylacetaldehyde, 3-methylbutanal, methional and 3-methylthiopropionaldehyde.^{25,30,38,43} Of
474 particular note are 2-methylbutanal, 3-methylbutanal and 3-methylthiopropionaldehyde which have been
475 shown to be the key determinants of worty off-flavors produced during CCF.¹⁵ These compounds diminish
476 even under CCF conditions to 60% below pre-fermentation values, but less so than when compared to
477 standard brewing methods.¹⁵ Other researchers reported compounds such as hexanal, 2,3-dimethylbutanol
478 and heptanal as also associated with worty off-flavors.¹⁶ Acetaldehyde in particular contributes to roughly
479 60–95% of the aldehyde content in beer and is useful as an analytical tracer.^{25,30} Aldehyde content is
480 influenced by factors such as fermentation contact period, temperature during fermentation, wort ventilation
481 and wort infection.³⁸ As far as storage considerations, 15% of Strecker aldehyde formation occurs here,
482 with the remainder derived from adducts during wort production.³⁰
483

484 Higher alcohols such as propanol and butanol are generally associated with negative flavor, though depend
485 heavily on concentration.^{25,38,42} Interestingly, these alcohols have been correlated with hangover effects.
486^{38,42} Of secondary consideration are sulphur compounds and organic acids. Sulphur compounds such as
487 DMS, sulphur dioxide and hydrogen sulphide are undesirable given the rotten egg flavor they are associated
488 with. They are noted as tolerable in smaller concentrations but not preferable except for the case of DMS,
489 which can improve the malt integrity of beer.³⁸ Finally, many contaminants can destroy a batch, such as
490 chlorophenols and bromophenols, originating from interactions with draught plastic tubing and
491 trichloroanisole which originates from damp and moldy environments.¹²
492
493

4.3 Positive Flavor Contributions

Though esters represent only a small portion of the composition of beer, they are extremely important.^{38,44} The most significant contributors to positive flavor profiles are ethyl acetate, isoamyl acetate, ethyl caproate, ethyl caprylate, ethyl hexanoate and phenyl ethyl acetate.^{12,25,44} Generally, ester production is influenced by fermentation temperature, wort aeration, attenuation limits, wort concentration and yeast strain, though it has been deduced as a function of all factors affecting yeast activity or substrate concentration.^{31,38} Most esters in beer are close to or just above the threshold levels implying minor processing changes can produce dramatic differences in taste.⁵⁴ Indeed, in the case of CCF and other L/AFB methods, high-gravity methods severely over-produce esters, resulting in excessive fruitiness.^{18,44} Furthermore, it has already been determined that anaerobic conditions and the absence of high levels of unsaturated fatty acids limit both cell growth and stimulate the production of acetate esters.²⁶

Though discussed previously as contributors to negative flavor profiles, some fusel alcohols and aldehydes deserve mention for their ability to contribute to a positive flavor profile, as well. These are propanol, isobutanol, 2-methylbutanal and 3-methylbutanal.²⁵ Of secondary consideration for positive flavor contributions are the nitrogen compounds and fatty acids produced by yeast during fermentation.³⁸ Examples of nitrogen compounds are amino acids and subsequently lower peptides, contributing to shape and palate roundness. Fatty acids lead to foamy and fatty flavors typically celebrated in ales and lagers and may have some importance with the ability to disguise negative flavors produced during CCF.³⁸

4.4 Flavor Thresholds

With the exception of synergistic flavor effects, a compound is considered detectable by taste once its concentration is higher than the compound flavor threshold. The lowest threshold that produces a stimulus is called the absolute or detection threshold.³⁰ From the absolute threshold, increasing the concentration of a substance will lead to the recognition threshold, allowing for identification. In the efforts of further standardization, the concept of flavor unit (FU) was introduced and is the ratio of the concentration of a flavor-active compound and its corresponding threshold value.³⁰ Heuristics have been recorded by professionals in the flavor field, such as that a 0.5 FU change can be perceived by a taster but defies identification, whereas a 1 FU change is sufficient for identification of the compound responsible.³⁰ Flavor thresholds for a large portion of the major flavor contributors have been compiled (Table 6).

Table 6 shows that aldehydes are of particular concern for brewers given their relatively low flavor thresholds relative to the other flavor contributors. As numerous authors have described previously, flavor thresholds can vary substantially given the subjective nature of evaluation methods as well as the type of matrix used, hence the variations in the table.

Table 6: Table detailing the flavor threshold values and ranges referenced in literature for the major flavor contributors detailed previously. Literature references are cited directly in the table.

Compound	Threshold (g L ⁻¹)	Reference	Flavor Association
<i>Esters</i>			
Ethyl acetate	(2.1 – 3.0)×10 ⁻²	44	Fruity, solvent-like
	(2.5 – 3.0)×10 ⁻²	34	
	3.0×10 ⁻²	33	
Isoamyl acetate	(0.6 – 1.2)×10 ⁻³	44	Banana, pear
	(1.2 – 2.0)×10 ⁻³	34	
	0.5×10 ⁻³	33	
Ethyl caproate	(0.17 – 0.21)×10 ⁻³	44	Apple, aniseed
	0.23×10 ⁻³	33	
Ethyl caprylate	(0.3 – 0.9)×10 ⁻³	44	Apple, sour apple
Phenyl ethyl acetate	3.8×10 ⁻³	44	Roses, honey
	(0.2 – 3.8)×10 ⁻³	34	
Ethyl hexanoate	(0.20 – 0.23)×10 ⁻³	34	Apple, pineapple
<i>Fusel alcohols</i>			
Propanol	6×10 ⁻¹	34	Solvent-like

Isobutanol	8×10^{-1}	33	
	1×10^{-1}	34	Solvent-like
	2×10^{-1}	33	
Isoamyl alcohol	$(5.0 - 6.5) \times 10^{-2}$	34	Solvent-like
<i>VDKs</i>			
Diacetyl	1.5×10^{-2}	45	Buttery, butterscotch
Pentane-2,3-dione	9×10^{-2}	45	Buttery
<i>Other Aldehydes</i>			
Acetaldehyde	1.1×10^{-3}	30	Green apple, fruity
	2.5×10^{-2}	30	
3-methylbutanal	6.0×10^{-4}	43	Malty, chocolate, cherry, wort
	5.6×10^{-5}	30	
2-methylbutanal	1.0×10^{-6}	43	Almond, apple-like, malty, wort
	4.5×10^{-5}	30	
<i>Trans</i> -2-nonenal	0.3×10^{-7}	30	Cardboard, papery, cucumber
	0.1×10^{-6}	30	
Furfural	1.5×10^{-1}	30	Caramel, bread, cooked meat
	1.5×10^{-2}	33	
3-methylthiopropionaldehyde	1.7×10^{-6}	43	Wort
<i>Secondary Contributors</i>			
DMS	$(0.3 - 1.0) \times 10^{-4}$	14	Cooked cabbage, sweet corn
Carbon dioxide	1	45	
Sulphur dioxide	2×10^{-5}	30	Striking-match

530

531 5. Mathematical Modeling and Simulation

532 Chemical processes are often dynamic in nature, whereby the amount of a chemical species can be either
533 increasing or decreasing with respect to time as a result of reactions and mass/energy flows in or out of the
534 system in question. This has led to the need for applying mathematical modelling to these systems, *i.e.* the
535 construction of a system of differential and algebraic equations, which seeks to describe a physical event
536 or process conceptually using mathematical language for the purposes of further manipulation and greater
537 insight. These mathematical models can be used for a broad range of purposes outside of engineering as
538 well, such as population-forecasting or ecological systems analysis.^{46,47} The constructed models are then
539 solved over the computational domain. This can be accomplished analytically to determine the exact
540 solution or by using numerical methods to estimate solutions arithmetically for systems that defy an exact
541 solution.⁴⁷ The numerical approach is required in some instances as the most general differential equation
542 is too difficult to solve directly (*i.e.* second order or higher) and a generalized solution may not yet exist for
543 the model. Historically, this has resulted in the extensive classification of differential equations and search
544 for analytical solutions to very specific problems as opposed to developing a general theory. This approach
545 has been demonstrated by some of the great mathematicians of the 17th and 18th century, such as Leonhard
546 Euler (1707–1783) with non-constant coefficient solutions, Jakob Bernoulli (1654–1705) with the Bernoulli
547 equation solution form, and Joseph-Louis Lagrange (1736–1813) with the parameter variation method.⁴⁸

548

549 5.1 Ordinary and Partial Differential Equations

550 The discussion of differential equations is vast. Thus, only a concise introduction into the topic is described
551 herein for the purpose of clarifying nomenclature and introducing general forms. As stated previously, the
552 mathematical formulation of problems encountered in engineering can lead to the generation of equations
553 involving derivatives of unknown functions.⁴⁸ These equations are known as differential equations and are
554 described generically in the ordinary, homogeneous and first-order form as:

555

$$F(x, y, y'(x)) = 0 \quad (2)$$

556

557 Derivatives are indicated using standard prime (') notation implying the relationship,

558

$$y'(x) = \frac{dy}{dx} \quad (3)$$

559
560 The order of the differential (e.g. first, second, third *etc.*) is denoted by the highest order derivative. This is
561 described more formally for an ordinary differential equation (ODE) as,
562

$$F(x, y(x), y'(x), y''(x), \dots, y^n(x)) = 0 \quad (4)$$

563 where F is said to be an n^{th} order differential equation on the unknown function $y(x)$ and prime notation is
564 used to describe the number of derivatives employed on the function $y(x)$. A differential equation is
565 classified as ordinary if it consists of ordinary derivatives with respect to a single independent variable.⁴⁸
566 An equation is described as a partial differential equation (PDE) if it consists of partial derivatives with
567 respect to two or more independent variables. A first order, homogeneous PDE is described as,
568
569

$$F(x_1, \dots, x_n; y, y'(x_1), \dots, y'(x_n)) = 0 \quad (5)$$

570
571 In addition, a discussion of linearity can be had, whereby an n^{th} order differential equation is considered
572 linear if it can be expressed as,
573

$$a_0(x)y^n(x) + a_1(x)y^{n-1}(x) + \dots + a_n(x)y(x) = f(x) \quad (6)$$

574 where $a_0(x), \dots, a_n(x)$ are functions of the variable x alone. In addition, if $f(x) = 0$ the differential
575 equation is said to be homogenous. Otherwise, the differential equation is described as inhomogeneous.⁴⁸
576
577

578 5.2 Differential-Algebraic Equation Systems and Solutions

579 Due to their dynamic nature, chemical processes can be modelled using differential-algebraic equation
580 (DAE) systems containing differential equations that describe the system with respect to mass and energy
581 balances and algebraic equations that ensure physical and thermodynamic relations between variables.⁴⁹
582 Mathematically, DAE systems are described as,
583

$$\mathbf{M}(x)\dot{\mathbf{x}} = \mathbf{f}(x) \quad (7)$$

584 where $\mathbf{M}(x)$ is a singular, state-dependent mass matrix, $\dot{\mathbf{x}}$ is a column vector comprised of differential and
585 algebraic equations for the system and $\mathbf{f}(x)$ is a column vector of algebraic equations. A system is described
586 as singular if there exist an infinite number of solutions, whereas a matrix is singular if its determinant is
587 zero.⁵⁰ These systems can be constructed and numerically simulated in software environments such as
588 MATLAB, where built-in first order numerical solvers such as 'ODE23' or 'ODE45' can be employed to
589 solve for and visualize the mathematical system variables.^{51,52} Higher-order DAE systems (of second or
590 greater order) can be solved for by substituting the higher-order ODEs with systems of a greater number of
591 first-order ODEs.⁵¹ The solver functions by applying direct numerical integration to the first-order DAE
592 using methods that are case- and solver- dependent. Some of the considerations include system stiffness,
593 whether the system is fully implicit, DAE differential index and the researcher's requirement for
594 computational expense/time savings.⁵¹
595
596

597 5.3 Stability and Sensitivity

598 Once a mathematical model has been constructed and verified, it is important to then evaluate it in terms of
599 its sensitivity and numerical stability. This analysis provides further understanding of the system and is a
600 necessary step prior to bioreactor optimization.⁵³ This is of particular importance for batch and semi-batch
601 operations, as they can exhibit very low sensitivity with respect to existing control policy.⁵³ Sensitivity S
602 refers to how the state variables are with respect to changes in the forcing parameters of the system.^{46,47}

603

$$S = \frac{\partial x}{x} / \frac{\partial P}{P} \quad (8)$$

604

605 where x is the state variable in question, P is the parameter being varied and ∂x and ∂P are the changes to
 606 either the state variable or parameter, respectively.⁵⁷ Here, the variations are denoted in terms of the
 607 italicized Latin letter ‘d’ (∂) in order to denote partial differentials, whereby the variables can be a function
 608 of several other independent variables of the system. In the context of chemical processing, large sensitivity
 609 values can provide an indication of which parameters need to be strictly controlled in order to prevent
 610 process deviations whereas low sensitivity values provide an indication of system inflexibility. It is
 611 important to note that the definition provided in equation (8) divides the changes ∂x and ∂P by x and P ,
 612 respectively. This provides improved relative numerical context when comparing between several
 613 parameter perturbations of varying size, as these divisions help to normalize the magnitude of the numerator
 614 and denominator. However, multiple definitions of sensitivity can be found that do not include these
 615 additional dividing terms.^{54,55} Sensitivity analyses can be either local or global in nature: Local analyses
 616 refer to small parameter perturbations, whereas global sensitivity analyses refer to the effects of large or
 617 simultaneous parameter changes on state variables.⁵⁵

618

619 Typically, when referring to sensitivity analyses, the finite difference/perturbation method is brought to
 620 mind, involving tedious re-simulations of inputs, parameter perturbations and measurement of the effects
 621 on state variables.⁵³ However, a sensitivity analysis can be extended to include the differential (derivative-
 622 based) method.^{53,55,56} The differential method focuses on investigating the effects of infinitesimally small
 623 changes to parameters via performance criteria, and is very algorithm- and computationally reliant.^{53,54}
 624 However, given that the basis for performing sensitivity analyses is rooted in developing further
 625 understanding of a system, it has been argued that the use of the derivative instead of the finite difference
 626 method may lead to misleading results with a premature understanding of the system. This is because
 627 researchers tend to have a better intuitive understanding of a system from the perspective of arithmetic
 628 difference of parameters than rate of change, especially when the system is nonlinear and time-dependent
 629 such as with batch chemical processing.⁵⁶

630

631 An understanding of the numerical stability of a system is also paramount. Stability is a function of the
 632 differential equation, the numerical method used to solve the differential equation and the step size used
 633 within the numerical calculation.⁵⁷ A numerical solution is considered stable if the rounding error remains
 634 small over the computational domain with respect to the exact solution.⁴⁸ This is best explained in generic
 635 terms through the concepts of relative error as derived from the first-order Taylor series approximation,
 636

$$f(x) = f(\tilde{x}) + f'(\tilde{x})(x - \tilde{x}) \quad (9)$$

637

638 where $f(x)$ denotes a generic function with respect to the variable x , \tilde{x} describes a variation from x due to
 639 numerical computation and $f'(\tilde{x})$ describes the first derivative of the function $f(\tilde{x})$ which is evaluated with
 640 respect to \tilde{x} .⁴⁷ Equation (9) can be rearranged to form an analogy for the relative error of $f(x)$,

641

$$\frac{f(x) - f(\tilde{x})}{f(x)} \cong \frac{f'(\tilde{x})(x - \tilde{x})}{f(x)} \quad (10)$$

642

643 By extension, the relative error of x is denoted as follows:

644

$$\frac{x - \tilde{x}}{\tilde{x}} \quad (11)$$

645
646 Finally, the condition number C_n is defined as,
647

$$C_n = \frac{\tilde{x}f'(\tilde{x})}{f(\tilde{x})} \quad (12)$$

648
649 The condition of a mathematical system is of great interest as it provides an indication of whether relative
650 errors (uncertainty) are magnified ($C_n > 1$), attenuated ($C_n < 1$) or identical ($C_n = 1$) to the relative error in a
651 state variable x .⁴⁷ An evaluation of C_n over a computational domain will reveal whether the condition
652 changes and by what amount. An unstable system would be one where the relative errors increase over the
653 computational domain (e.g. time domain with respect to a batch chemical reaction) and a stable system is
654 one where the relative errors decrease or remain the same over the computational domain. Functions with
655 large condition numbers are described as ill-conditioned, and systems that are close to being singular are
656 often ill-conditioned.⁴⁷ The combined consideration of sensitivity, stability and condition provide a clearer
657 picture in regard to the quantitative robustness of the system. The real value of these concepts extends into
658 industrial applications, where model robustness equates to more accurate predictions of performance with
659 respect to inevitable changes in parameters and processing conditions. This results in less process down-
660 time as a result of troubleshooting or uncertainty of outcome.

661 662 **6. Fermentation Modeling and Parameterization**

663 The reaction mechanisms related to enzyme kinetics, most notably Michaelis-Menten kinetics, have existed
664 for over a century as a mathematical tool to describe the formation of a product (P) resulting from the
665 enzymatic (E) linking with a substrate (S). A typical form of an enzymatic reaction is formulated as,
666



667
668 where k_2 and k_3 describe the rate constant for the corresponding forward reactions at either step and k_1
669 describes the rate constant for the reverse reaction. The intermediate substrate bound to the enzyme is
670 denoted as SE . One can arrive at an expression for the rate of product formation (r_p) as,
671

$$r_p = \frac{k_3 C_S C_E^0}{K_M + C_S} \quad (14)$$

672
673 where C_S and C_E^0 are the substrate and initial enzyme concentrations, respectively. The variable K_M is the
674 Michaelis-Menten constant which is equal to the ratio k_1/k_2 . Though enzymes are lifeless chemical
675 substances produced by yeast to catalyze chemical reactions, organisms which grow (*i.e.* towards biomass
676 production) can be described slightly differently. A mathematical formula that can be used to describe the
677 activities of organisms such as yeast is the Monod equation,
678

$$r = \frac{r_m C_S}{K_S + C_S} \quad (15)$$

679
680 where r is the specific growth rate of biomass, r_m is the maximum specific growth rate of biomass and K_S
681 is the Monod constant.⁵⁸ Despite the formulation of these mechanisms so long ago, the application of kinetic
682 modelling to the entire beer fermentation process in a computational context is a relatively recent endeavor,
683 beginning with the first computational kinetic modelling of beer fermentation in 1981.⁵⁹ However, in the
684 context of CCF, since the first mention of CCF in 1983, the instances of CCF assays in literature have
685 remained experimental in nature.^{15, 17-18, 20-21, 25-27, 35, 40} In the interest of providing historical context to the
686 mathematical modelling and optimization of the beer fermentation process, a chronological timeline of the

687 most important published works since 1981 has been compiled (Table 7). Other published works that are
 688 similar in scope or content exist. However, they have been omitted as being of secondary impact in
 689 comparison to those listed in Table 7.
 690

Table 7: Table detailing the chronological timeline of mathematical modelling and optimization with respect to the fermentation process along with the first instance of CCF in literature. M = Important Mathematical Model, O = Optimization Study, E = Experimental Study, C = Fermentation Control Study. Improvements from previous to subsequent studies are listed in the “Context/Improvements” column.

Author	Year	Tag	Context/improvements
Engasser, Marc, Moll, <i>et al.</i> ⁵⁹	1981	M	First kinetic model of beer fermentation
Schur ¹⁸	1983	E	First publishing of CCF process, conditions
Stassi <i>et al.</i> ⁶⁰	1987	C	CO ₂ rate correlated with fermentation rate
Gee & Ramirez ⁶¹	1988	O	From Engasser <i>et al.</i> 1981 ⁵⁹ : added temperature effects, removed yeast flocculation, removed flavor model
Garcia, Garcia & Diaz ⁶²	1994	M	Kinetic model for the production of diacetyl
Gee & Ramirez ⁴²	1994	M ₁	From Gee, Ramirez 1988 ⁶¹ : Adjusted ethanol production, Arrhenius dependency, added CO ₂ generation, amino acid, inhibition and flavor models
Gee & Ramirez ⁶³	1996	C	Various algorithms for parameter estimation
De Andrés-Toro <i>et al.</i> ⁶⁴	1997	O	Genetic algorithm for fermentation optimization based on temperature profile
De Andrés-Toro <i>et al.</i> ⁶⁵	1998	M	From Gee, Ramirez 1994 ⁴² : Biomass segregated into lag, active and dead cells, sugars consolidated to one sub-model, flavor model reduced to just diacetyl (as in Garcia <i>et al.</i> 1994 ⁶²) and ethyl acetate
Corrieu, Trelea & Perret ⁶⁶	2000	C	From Stassi <i>et al.</i> 1987 ⁶⁰ : Incorporated on-line density estimation/prediction
Titica <i>et al.</i> ⁶⁷	2000	E	From Corrieu <i>et al.</i> 2000 ⁶⁶ : Modelled kinetics of fusel alcohols and esters from CO ₂ emissions
Trelea <i>et al.</i> ⁶⁸	2001	M	From Gee, Ramirez 1994 ⁴² and de Andres-Toro <i>et al.</i> 1998 ⁶⁵ : Predictive modelling is improved with CO ₂ emission-based models given industrial applicability. Adjusted all models.
Kurz ⁶⁹	2002	M	Metabolic and Black-Box Models for <i>Saccharomyces sp.</i> propagation
Carillo-Ureta ³⁸	2003	O	From de Andres-Toro <i>et al.</i> 1998 ⁶⁵ , Gee, Ramirez 1994 ⁴² and Garcia <i>et al.</i> 1994 ⁶² : Included some of these models towards control optimization with additional experimental parameters.
De Andrés-Toro, Giron-Sierra & Fernandez-Blanco ⁷⁰	2004	O	From de Andres-Toro <i>et al.</i> 1998 ⁶⁵ : Pareto approach with multi-objective evolutionary algorithms, redefined ethyl acetate growth
Xiao, Zhou, Zhang ⁷¹	2004	O	From e Andrés-Toro <i>et al.</i> 1998 ⁶⁵ : Use of ant colony (stochastic) algorithm for optimization, omission of ethyl acetate profiles.
Roeva ⁷²	2005	O	Comparing genetic algorithms for estimation
Ramirez & Maciejowski ⁷³	2007	O	From Gee, Ramirez 1994 ⁴² : Used model with sequential quadratic programming for optimization
Bosse & Griewank ⁷⁴	2014	O	From Gee, Ramirez 1994 ⁴² and de Andrés-Toro <i>et al.</i> 1998 ⁶⁵ : Optimal control with Lipschitz-constraint
Rodman & Gerogiorgis ¹³	2016a	M ₁	From de Andrés-Toro <i>et al.</i> 1998 ⁶⁵ : diacetyl and ethyl acetate parameters redefined
Rodman & Gerogiorgis ⁷⁵	2016b	O	From Rodman, Gerogiorgis 2016a ¹³ : Added process condition variation to visualization
Rodman & Gerogiorgis ⁷⁶	2016c	O	From Rodman, Gerogiorgis 2016a ¹³ : Sensitivity analysis and dynamic optimization for flavor
Rodman, Fraga & Gerogiorgis ²⁸	2018	O	From Rodman, Gerogiorgis 2016a ¹³ : Optimization using stochastic evolutionary algorithm
Rodman & Gerogiorgis ⁷⁷	2019	O	From Rodman, Gerogiorgis 2016a ¹³ : Optimization comparison - Control Vector Parameterization and Complete Parameterization

691
 692 A thorough review of the literature compiled in Table 7 has led to the selection of two main bodies of work
 693 that have been used for simulating fermentation, albeit under non-CCF conditions. These selections were
 694 made given the manner in which researchers have built upon the work of others. The only models
 695 considered hereafter have tags with the subscript ‘1’ in Table 7. The models from these works have been
 696 subdivided into the Growth Model, the Amino Acid Model and the Flavor Model. Parameters for these
 697 models can be found in their respective papers. Parameters that are derived experimentally are preferred,

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as well as those which are non-isothermal. As previous modelling has only been performed under non-CCF conditions, explicit CCF parameters are not available and require extrapolation or novel estimation studies.

Table 8: Table of relevant models part of the Growth Model, retrieved from literature (Table 7) [*: only portion of the full model]

Sub-Model	Equations (Gee & Ramirez, 1994) ⁴²	Equations (Rodman & Gerogiorgis, 2016a) ¹³
Biomass Production	$\frac{dX}{dt} = \mu_x \cdot X = [Y_{XG}\mu_1 + Y_{XM}\mu_2 + Y_{XN}\mu_3]$ (16)	$\frac{dX_A}{dt} = \mu_x \cdot X_A - \mu_{DT} \cdot X_A + \mu_L \cdot X_L$ *(17) $\mu_x = \frac{\mu_{x0} \cdot C_S}{k_x + C_e}$ *(18)
Ethanol Production	$E = E_0 + Y_{EG}(G_0 - G) + Y_{EM}(M_0 - M) + Y_{EN}(N_0 - n)$ (19)	$\frac{dC_E}{dt} = f \cdot \mu_e \cdot X_A$ (20) $\mu_e = \frac{\mu_{e0} \cdot C_S}{k_e + C_s}$ (21)
Glucose Consumption	$\frac{dG}{dt} = -\mu_1 \cdot X$ (22)	$\frac{dC_S}{dt} = -\mu_S \cdot X_A$ (23)
Maltose Consumption	$\frac{dM}{dt} = -\mu_2 \cdot X$ (24)	-
Maltotriose Consumption	$\frac{dN}{dt} = -\mu_3 \cdot X$ (25)	-
Glucose Specific Growth Rate	$\mu_1 = \frac{\mu_G G}{K_G + G}$ (26)	$\mu_S = \frac{\mu_{s0} \cdot C_S}{k_s + C_e}$ (27)
Maltose Specific Growth Rate	$\mu_2 = \frac{\mu_M M}{K_M + M} \cdot \frac{K'_G}{K'_G + G}$ (28)	-
Maltotriose Specific Growth Rate	$\mu_3 = \frac{\mu_S N}{K_N + N} \cdot \frac{K'_G}{K'_G + G} \cdot \frac{K'_M}{K'_M + M}$ (29)	-
Temperature dependency	$\mu_i = \mu_0 \exp[-E\mu_i/RT^2], i = G, M, N$ (30)	$\mu_{i0} = \exp(A_i + \frac{B_i}{T})$ (33)
	$K_i = K_{i0} \exp[-E_{ki}/RT^2], i = G, M, N$ (31)	
	$K'_i = K'_{i0} \exp[-E'_{ki}/RT^2], i = G, M$ (32)	
Fermenter Temperature	$\frac{dT}{dt} = \frac{1}{\rho C_p} [-X(\Delta H_{FG}\mu_1 + \Delta H_{FM}\mu_2 + \Delta H_{FN}\mu_3) - u(T - T_c)]$ (34)	-
CO ₂ Liquid Phase	$\frac{dC_l}{dt} = \begin{cases} K_{GL}(C_{sat} - C_l) & \text{for } C_l < C_{sat} \\ 0 & \text{for } C_l = C_{sat} \end{cases}$ (35)	-
CO ₂ Gas Phase	$\frac{dC_g}{dt} = \begin{cases} (Y_{CG}\mu_1 + Y_{CM}\mu_2 + Y_{CN}\mu_3)X & \\ -K_{GL}(C_{sat} - C_l) & \text{for } C_l < C_{sat} \\ (Y_{CG}\mu_1 + Y_{CM}\mu_2 + Y_{CN}\mu_3)X & \\ \text{for } C_l = C_{sat} & \end{cases}$ (36)	-
Inhibition	$\mu_x = (Y_{XG}\mu_1 + Y_{XM}\mu_2 + Y_{XN}\mu_3) \frac{K_x}{K_x + (X - X_0)^2}$ (37)	$f = 1 - \frac{C_e}{0.5 \cdot C_0}$ (38)

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6.1 Growth Model

The Growth Model here is characterized as the combination of any models in literature representing sugar consumption, biomass production, ethanol production, temperature effects and the release of carbon dioxide. Of great importance to cell growth are the several sugars available in the brewer's wort. The three main sugars that wort is comprised of are glucose (10–15%), maltose (50–60%) and maltotriose (15–20%).¹¹ Glucose is preferentially used by yeast in comparison to maltose and maltotriose, though full process efficiency (fully utilizing the fermentable extract) requires the complete fermentation of all three sugars.¹¹ However under CCF conditions, glucose repression of the genes responsible for uptake claim partial responsibility for the incomplete and slower consumption for maltose and maltotriose, possibly resulting in

712 higher caloric content and negative flavor associations in beer in general.^{11,21} Studies have also shown that
 713 a step-wise approach to implementing both anaerobic and aerobic conditions leads to an optimal and
 714 constant flavor profile in AFB, as well as allowing for constant cell growth.²⁶ A compilation of the models
 715 pertaining to sugar consumption as well as the remaining elements of the Growth Model are shown below
 716 (Table 8). Information detailing variables can be found in the original papers.

717
 718 As seen in Table 8, some authors have preferred to consolidate all sugars into one sub-model. Inhibition,
 719 temperature dependencies, biomass growth and ethanol consumption are all re-formulated and carbon
 720 dioxide emissions and changes to fermenter temperature have been omitted in some models. Of note is the
 721 differences between biomass growth models, with some authors preferring to separate growth into lag,
 722 active and dead cells, as well as a transitioning between a lag and a fermentation phase.⁶⁵

723 6.2 Amino Acid Model

724
 725 The Amino Acid Model consists of equations indicating the consumption of amino acids such as leucine,
 726 isoleucine and valine towards the consumption of flavor compounds such as fusel alcohols (Table 9).⁴ As
 727 seen in Table 9, work by Gee and Ramirez (1994) included the consumption of relevant amino acids,
 728 whereas other authors chose not to include them in their model in the interest of simplicity.⁴²

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Table 9: Table of relevant models part of the Amino Acid Model, retrieved from literature (Table 7).		
Sub-Model	Equations (Gee, Ramirez 1994) ⁴²	Equations (Rodman, Gerogiorgis 2016a) ¹³
Leucine Uptake	$\frac{dL}{dt} = -Y_{lx} \cdot \frac{dX}{dt} \cdot \frac{L}{K_L + L} \left(1 - \exp\left(-\frac{t}{\tau_d}\right)\right)$ (39)	-
Isoleucine Uptake	$\frac{dI}{dt} = -Y_{Ix} \cdot \frac{dX}{dt} \cdot \frac{I}{K_I + I} \left(1 - \exp\left(-\frac{t}{\tau_d}\right)\right)$ (40)	-
Valine Uptake	$\frac{dV}{dt} = -Y_{Vx} \cdot \frac{dX}{dt} \cdot \frac{V}{K_V + V} \left(1 - \exp\left(-\frac{t}{\tau_d}\right)\right)$ (41)	-

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 733 **6.3 Flavor and Aroma Model**
 734 The production of secondary flavor compounds has also been taken into consideration (Table 10). As seen
 735 in Table 10, work by Rodman and Gerogiorgis represents a reduced version of modelling of flavor products,
 736 choosing to include only the ethyl acetate and diacetyl formation in the fermenter.¹³ Other compounds are
 737 not included in either model, such as free sulphur dioxide which disappears in beer over time at a very low
 738 rate at 0°C and faster at higher temperature following first-order kinetics.³⁰

739 6.4 A Computational Perspective

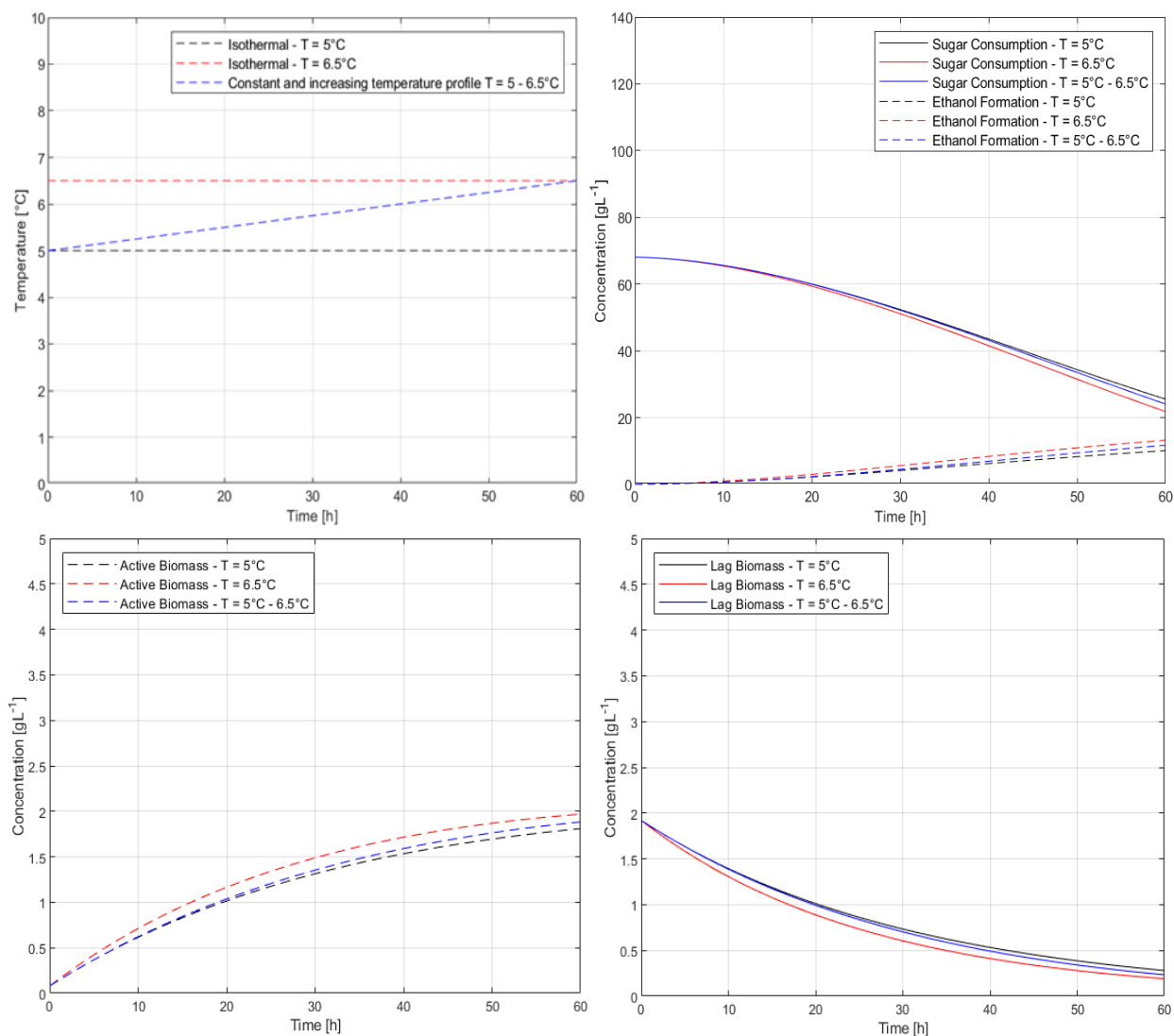
740
 741 A computational implementation of the de Andrés-Toro et al. (1998) model⁶⁵ has been undertaken in order
 742 to trace and visualize the key state variables (sugar, ethanol, biomass) for prospective CCF implementation.
 743 The initial conditions and plausible temperature profiles must be carefully selected in order to reliably
 744 replicate industrial CCF operation; parameter values used should preferably be validated at least against
 745 final-time CCF experimental results (details beyond our scope here form part of a forthcoming submission).
 746
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748 To evaluate how previously validated parameter values of the de Andrés-Toro et al. model ($T=13$ °C)^{13,65}
 749 affect model accuracy for CCF operation, we consider three different (two isothermal and one ascending)
 750 temperature manipulation profiles, and perform dynamic simulations of key output trajectories (Figure 12).
 751 Sugar consumption advances significantly but remains incomplete in the time horizon explored ($t = 60$ hrs).
 752 Even at a lower initial sugar concentration, attenuation (ensuring no residual sugar) requires a few days.

753 Considering three different fermentor temperature profiles illustrates the extreme sensitivity of CCF to
 754 brewing conditions: the final sugar concentration, $C_S(t = 60 \text{ hrs})$, varies by 5.7% between the lower and
 755 the higher isothermal profile (as expected, the higher $T = 6.5 \text{ }^\circ\text{C}$ profile expedites biochemical phenomena).
 756

757 Ethanol production is to be suppressed in CCF; indeed, $C_E(t)$ it rises much slower than the $T = 13 \text{ }^\circ\text{C}$ case.
 758 Parameter estimation accuracy is critical to accurately compute final ethanol concentration: the plot shows
 759 it is significantly reduced under these CCF conditions, albeit $C_E(t = 60 \text{ hrs}) < 5 \text{ g}\cdot\text{L}^{-1}$ is often desirable.
 760 For ethanol, the effect of temperature manipulation profile variation is more pronounced: the final ethanol
 761 concentration, $C_E(t = 60 \text{ hrs})$, varies by 16% for a mere $\Delta T = 1.5 \text{ }^\circ\text{C}$ between the two isothermal profiles.
 762

763 Active $X_A(t)$ and lag $X_L(t)$ biomass evolution are also two state variables of importance for CCF runs.
 764 Higher temperatures clearly facilitate the proliferation of the former at the expense of the latter (Figure 12);
 765 in the considered initial conditions, we employ the standard assumption of $X_A(t = 0) \ll X_L(t = 0)$ ¹³.
 766 Consequently, temperature manipulation profile variation affects lag more than active biomass at final time:
 767 while $X_A(t = 60 \text{ hrs})$ varies by 4.0%, $X_L(t = 60 \text{ hrs})$ varies by 16.6% between the isothermal profiles.
 768 Remarkably, active biomass evolution is much slower in CCF than in standard ($T = 13 \text{ }^\circ\text{C}$) fermentation.
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Figure 12. Sugar, ethanol, active and lag biomass responses for three plausible CCF temperature profiles.

774 **7. Critical Review**

775 Several concepts are illuminated with respect to the literature surveyed for this study. The foremost concern
 776 relates to the asymmetrical balance of references with respect to standard alcoholic beer and those available
 777 for AFB, of which CCF is just a small part. Relatively speaking, there is an abundance of studies making
 778 use of physical/post-processing methods for dealcoholization, which outnumber biological/pre-processing
 779 methods. Literature pertaining to CCF is very limited in comparison, with few assays referenced and only
 780 a handful of lab scale endeavors. The present review does not cover the patent literature on production of
 781 L/AFB, from where substantial knowledge could be gathered. These two points should be carefully
 782 considered by industrial corporations before implementing or improving CCF methods, as batch operations
 783 are notorious for being difficult to scale-up from bench-top studies. Pre-processing methods not only appear
 784 to be the more preferable option on paper: they have also been cited as more common, as post-processing
 785 methods require extra capital expenditure, making them less attractive to brewers. Therefore, a large portion
 786 of the knowledge available for the production of AFB through CCF/pre-processing methods seems to be
 787 available as trade secrets and/or plant rules of thumb developed by experienced brewing professionals, as
 788 increasing L/AFB sales indicate that flavor-balanced, palatable products have already been manufactured.
 789

Table 10: Table of relevant models part of the Flavor and Aroma Model, retrieved from literature (Table 7).

Sub-Model	Equations (Gee, Ramirez 1994) ⁴²	Equations (Rodman, Gerogiorgis 2016a) ¹³
Isobutanol Production	$\frac{d[IB]}{dt} = Y_{IBE}\mu_V X$ (42)	–
Isoamyl alcohol Production	$\frac{d[IA]}{dt} = Y_{IAE}\mu_L X$ (43)	–
2-methyl-1-butanol Production	$\frac{d[MB]}{dt} = Y_{MBE}\mu_I X$ (44)	–
Ethyl acetate Production	$\frac{d[EA]}{dt} = Y_{EAS}[\mu_1 + \mu_2 + \mu_3]X$ (45)	$\frac{dC_{EA}}{dt} = Y_{EA} \cdot \mu_x \cdot X_A$ (46)
Ethyl caproate Production	$\frac{d[EC]}{dt} = Y_{ECX}\mu_x X$ (47)	–
Isoamyl alcohol Production	$\frac{d[IAC]}{dt} = Y_{IAC}\mu_{IA} X$ (48)	–
Propanol Production	$\frac{d[P]}{dt} = Y_{PE}[\mu_V + \mu_I]X$ (49)	–
Diacetyl Production	$\frac{d[VDK]}{dt} = Y_{VDK}\mu_x X - k_{VDK}[VDK]X$ (50)	$\frac{dC_{DY}}{dt} = \mu_{DY} \cdot C_S \cdot X_A + \mu_{AB} \cdot C_{DY} \cdot C_E$ (51)
Acetaldehyde Production	$\frac{d[AAI]}{dt} = Y_{AAI}[\mu_1 + \mu_2 + \mu_3]X - k_{AAI}[AAI]X$ (52)	–

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 791 The synergistic or suppressive effects of flavor represent a double-edged sword as well, as incomplete
 792 knowledge of the sensorial interactions of a mixture of compounds could lead to counter-productive results
 793 in the instances where flavor active compounds are added to beer prior to bottling or when used as system
 794 production constraints. By extension, in implementing mathematical modelling, thresholds can be
 795 analogously used as limiting constraints, as previous research has shown. However, the implementation of
 796 a buffer between these thresholds should be considered to prevent a synergistic effect enhancing a negative
 797 flavor compound beyond the constraint. As a final note on organoleptic properties, no evaluation of aroma
 798 is present in this review under the assumption that it will have a less significant effect on the product appeal
 799 and is already implicit in the discussion when evaluating flavors.

800
 801 Not all mathematical models are considered equal. As cautioned by researchers, over-parameterization or
 802 the use of more sub-models than can be validated experimentally is neither pragmatic nor valuable.
 803 However, over-generalization, though useful for eliminating costly experimental validation or
 804 computational cost, can provide a simplistic result that is blind to the fundamental issues. In the case of

805 CCF, flavor is of highest concern and so future work should ensure accurate modelling of key culprits for
806 the negative flavor profiles so long as they are not tied simplistically to mere fermentation progression.

807
808 Of the biological options available currently to produce AFB, the most promising options are CCF with
809 free mass yeast or CCF with immobilized yeast given their ability to meet very low alcohol specifications
810 without the requirement of additional post-processing equipment. Though difficult to control, they are
811 arguably no more difficult than the current batch methods that the entire brewing industry is founded on,
812 manufacturing an enormous amount of flavorful and balanced products worth billions of dollars a year.

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814

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823

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