PREDICTION OF BREWERY SPENT GRAIN DEGRADATION KINETICS AND OPTIMIZATION OF CRITICAL PARAMETERS TO ENSURE THEIR STABILITY

POLIKSENI DRAZHO[1], LULJETA PINGULI[2], ROZANA TROJA[3], ILIRJAN MALOLLARI[4], TERKIDA PRIFTI[5]

[1]Department of Risk Assessment, Albanian National Food Authority,

Tirana, Albania

[2,3,4,5] Faculty of Natural Sciences, Department of Industrial Chemistry, University of Tirana, Albania

e-mail : polidrazho@outlook.com;

Abstract

Predicting the degradation kinetics of Brewery Spent Grain (BSG) is crucial for optimizing preservation strategies and minimizing quality loss during storage. Key environmental parameters, such as temperature, pH, and moisture content, significantly influence the degradation rate by affecting microbial activity and biochemical stability. Temperature is one of the primary physical parameters that can either promote or inhibit the rate of biochemical reactions, thereby influencing microbial activity. In this context, the classical Arrhenius model was applied to predict the effect of temperature on biochemical reaction rates. The environmental pH also plays a critical role in BSG preservation. It can act as either an inhibitor, or an activator of enzymatic and microbial processes, particularly influencing the development of pathogenic microorganisms. One of the most critical characteristics of fresh, untreated BSG is its high moisture content and water activity, which create ideal conditions for microbial proliferation. The degradation kinetics of BSG is analyzed using well-established microbiological models, incorporating the effects of temperature, pH, and moisture to make mathematical predictions. Accurately modeling degradation kinetics helps identify optimal environmental conditions to minimize BSG degradation. These models hold significant value in engineering applications, as they can be integrated into automated control systems that utilize sensors to continuously monitor environmental parameters. Integrating mathematical models with

experimental data, enables the development of effective strategies to slow degradation, enhance efficiency, and preserve product quality.

Key words: *Brewery Spent Grain (BSG), degradation, prediction, mathematical models (Arrhenius model), microbiological models, stability.*

Përmbledhje

Parashikimi i kinetikës së degradimit të Bërsive të Mbetura të Birrës (BSG) është thelbësor për optimizimin e strategjive të ruajtjes dhe minimizimin e humbjes së cilësisë gjatë magazinimit. Parametrat kryesorë mjedisorë, të tillë si temperatura, pH dhe lagështia, ndikojnë ndjeshëm në shkallën e degradimit për shkak të efektit në aktivitetin mikrobial dhe stabilitetin biokimik. Temperatura është një nga parametrat kryesorë fizikë që mund të nxisë ose të pengojë reaksionet biokimike, duke ndikuar kështu në aktivitetin mikrobial. Në këtë kontekst, për të parashikuar ndikimin e temperaturës në shpejtësinë e reaksioneve biokimike është zbatuar modeli klasik i Arrhenius-it. PH mjedisor luan gjithashtu një rol kritik në ruajtjen e BSG. Ai mund të veprojë si një inhibitor ose aktivizues i proceseve enzimatike dhe mikrobiale, duke ndikuar veçanërisht në zhvillimin e mikroorganizmave patogjenë. Një nga karakteristikat më kritike të BSG të freskët dhe të patrajtuar është përmbajtja e lartë e lagështisë dhe aktiviteti i ujit, të cilat krijojnë kushte ideale për shumimin mikrobial. Kinetika e degradimit të BSG analizohet duke përdorur modele të mirëpërcaktuara mikrobiologjike, në të cilat përfshihen efektet e temperaturës, pH dhe lagështisë, për qëllime të parashikimeve matematikore. Modelimi i saktë i kinetikës së degradimit, ndihmon në identifikimin e kushteve optimale mjedisore për të minimizuar degradimin e BSG. Këto modele kanë vlerë në aplikimet inxhinierike, pasi mund të integrohen në sistemet e automatizuara të kontrollit, duke përdorur sensorë për monitorimin e vazhdueshëm të parametrave mjedisorë. Integrimi i modeleve matematikore me të dhënat eksperimentale, mundëson zhvillimin e strategjive efektive për të ngadalësuar degradimin, përmirësuar efikasitetin dhe ruajtjen e cilësisë së produktit.

Fjalë kyçe: *Bërsitë e mbetura të birrës (BSG), degradim, parashikim, model matematikor (modeli Arrhenius), model mikrobiologjik, stabilitet.*

Introduction

Brewery spent grain (BSG) is the primary by-product of beer production, accounting for approximately 85 % of the total waste generated during the brewing process (Mussatto et al., 2006; Kitaw et al., 2022). For every 100 liters

of beer produced, around 20 kg of fresh BSG is generated, which translates into over 36 million tons annually on a global scale (Mussatto et al., 2006; Kitaw et al., 2022). Although BSG is rich in dietary fiber and proteins, its high moisture content renders it highly perishable, leading to rapid spoilage (Kitaw et al., 2022). Studies indicate that BSG with 70 – 80 % moisture can spoil within 2 – 7 days under ambient conditions because of the fast growth of microorganisms that feed on its polysaccharides and proteins (Mussatto et al., 2006). If not properly preserved, BSG loses dry matter and nutritional value, emits off-odors, and may develop fungal growth that produces hazardous toxins, such as aflatoxins and ochratoxin A (Mussatto et al., 2006; Kitaw et al., 2022).

The high moisture content is the main driver of spoilage. Fresh BSG typically contains about 70 – 80 % water, corresponding to a water activity (a_w) above 0.95 (Mussatto et al., 2006). Such a high a_w creates an ideal environment for bacteria, yeasts, and molds to proliferate (Bourdoux et al., 2016). In contrast, most bacteria cannot grow in foods with a_w below 0.87, and yeasts and molds are generally inhibited when a_w falls below 0.60 (Bourdoux et al., 2016). Therefore, it is essential to drastically reduce the moisture content, ideally below 10%, to prevent microbial growth. Drying is the most used preservation method. Many breweries first dewater BSG (by pressing or centrifugation) to reduce moisture to around 50 – 60 % and then dry it with hot air until the moisture content is below 10 % (Ashbell et al., 2002).

Temperature plays a significant role in influencing spoilage rates. Elevated temperatures accelerate biochemical reactions and microbial growth according to an Arrhenius-type relationship (Cohen, 1985). Lower temperatures, on the other hand, markedly slow these processes. For example, one study demonstrated that BSG stored under aerobic conditions at 15 °C showed no visible spoilage for up to 6 days, whereas at 20 °C, slight mold growth began after 4 days and became pronounced by day 6 (Kitaw et al., 2022). In practical terms, microbial counts can rise from acceptable initial levels to over 5 $\log_{10}$ CFU/g – a threshold considered unsafe, within a few days at temperatures above 15 °C (Wang et al., 2014).

The pH of the BSG environment is another critical factor. Fresh BSG typically has a mildly acidic pH, ranging from 4.5 to 6.0, because of the brewing process (Hermansen et al., 2024). Under aerobic conditions, two trends may emerge: first, native lactic acid bacteria can ferment residual sugars, further lowering the pH; and second, proteolytic degradation by molds can release alkaline

compounds that eventually raise the pH (Hermansen et al., 2024). Maintaining an acidic environment (preferably below pH 4.2) is therefore crucial to inhibit spoilage microorganisms. This principle underpins ensiling (anaerobic fermentation), where rapid lactic acid production lowers the pH to below 4.0, thus preventing the growth of undesirable organisms (Terefe, 2022).

Predictive models are essential for forecasting the stability of BSG under varying conditions. Exponential and logistic microbial growth models are vital; as microbial proliferation is the primary driver of BSG spoilage. Therefore, accurately modeling these dynamics is essential for predicting degradation patterns. Initially, when nutrients are abundant, microorganisms typically exhibit exponential growth. However, once exposed to air, environmental microorganisms quickly colonize the substrate. Following a brief lag phase, these organisms enter an exponential phase of growth. The resulting growth curve is typically sigmoidal (logistic), encompassing a lag phase, an exponential phase, and a plateau when nutrients become limiting or metabolic by-products accumulate (Ashbell et al., 2002). For BSG, this pattern is clearly observed: at lower temperatures (15 °C), microbial populations remain relatively stable over several days, while at higher temperatures (20–25 °C) the exponential phase is reached within about 2 days before leveling off or declining as nutrients are depleted (Kitaw et al., 2022; Wang et al., 2014). This logistic growth is directly correlated with spoilage kinetics, as the most significant quality changes, such as organic matter loss, protein degradation, and toxin production, occur during the exponential phase (Kitaw et al., 2022).

Methodology

The physicochemical characterization of brewer's spent grain (BSG) was conducted utilizing the laboratory facilities at the "Stefani & Co" brewery in Tirana, Albania. Microbiological analyses were in part conducted on-site at the brewery, while additional analyses were conducted in the microbiological laboratory at the Faculty of Natural Sciences, Department of Industrial Chemistry. Fresh BSG samples were collected immediately post-production. Two dilutions were prepared; 10 g fresh BSG diluted in 90 mL sterile H□O (1:10 dilution) and 10 g fresh BSG diluted in 190 mL sterile H□O (1:100 dilution).

The samples were mixed well and let to rest for 20 minutes. Microbial cultivation involved: Pour plate technique using Plate Count Agar and Spread Plate Technique using Wort Agar. Cultures were incubated over several consecutive days to monitor microbial succession and assess changes in

microbial populations associated with the aging of BSG during storage. The samples were stored at environmental laboratory conditions (18 °C).

Sampling intervals included: **Day 0:** Fresh BSG; **Day 1:** 1-day-old BSG; **Day 2:** 2-day-old BSG; **Day 6:** 6-day-old BSG and **Day 8:** 8-day-old BSG.

The kinetic constants were determined using classical biochemical engineering equations, as outlined in the book on *Biochemical process engineering* (Pinguli, Malollari 2022).

Table 1. A summary of the applied equations.

Description of the equation	Used equation	Equation Number
Equation of the rate of cell mass production	$\frac{dN}{dt} = \mu \times N$; with initial conditions $N\|_{t=t_L} = N$ $ln\frac{n}{n_0} = \mu(t - t_L)$ $N(t) = N_0 e^{\mu t}$	(1)
Equation of the cell doubling time	$t_d = \frac{ln\,2}{\mu}$	(2)
Equation of the Logistic Growth Curve	$\mu = \mu_{max} N\left(1 - \frac{N}{N_{max}}\right)$ Integrated form: $N = \frac{N_{max}}{1 + \left(\frac{N_{max} - N_0}{N_0}\right) e^{-\mu_{max.t}}}$	(3)
Arrhenius Equation (Temperature Dependence):	$\mu = A_a \exp\left(-\frac{E_a}{R \times T}\right) = k(T)$	(4)
Linearized form of the Arrhenius equation	$ln\,\mu = ln\,A_a - \frac{E_A}{R} \times \frac{1}{T}$	(5)

Modification of the rate constant by water activity	$k_{effect} = k_{opt} \cdot f(a_w); \qquad f(a_w) = \frac{a_w - a_{w,min}}{1 - a_{w,min}}$ $N(t) = N_0 \cdot e^{-k_{effect} \cdot t} = N_0 \cdot e^{-k_{opt} \cdot \frac{a_w - a_{w,min}}{1 - a_{w,min}} \cdot t}$	(6)
Modification of the rate constant by temperature	$N(t) = N_0 e^{k(T)t}$	(7)
Modification of the rate constant by pH	$k_{effect} = k_{opt} \cdot f(pH)$ $k(pH) = k_{opt} \cdot \left(1 - \left\lvert \frac{ph - pH_{opt}}{pH_{max} - pH_{min}} \right\rvert^n \right)$	(8)
Modification of the rate constant considering temperature, pH and water activity.	$k_{effect} = k_{opt} \cdot f(T) \cdot f(pH) \cdot f(a_w)$	(9)
Exponential model for microbial growth	$N = N_0 \cdot e^{\mu t} \Longrightarrow t = \frac{\ln(N) - \ln(N_0)}{\mu}$	(10)

Calculation Procedure

Table 2 presents a summary that indicates the maximum levels of microorganisms in Spent Brewery Grain according to the recommendations of several important institutions (authorities/regulators). These values are based on general guidelines for food safety and the hygiene of food products, such as the guidelines from EFSA (European Food Safety Authority), FDA (U.S. Food and Drug Administration), and Codex Alimentarius (the Food Codex Commission). Precise standards vary depending on local regulations, the intended specific use (animal feed, bioenergy, or other food products), and country-specific guidelines.

Table 2. Maximum allowed levels of microorganisms in Spent Brewery

Microorganism	**Maximum allowed level**
Total bacteria (Total Plate Count)	≤ 10□ - 10□ CFU/g
Enterobacteriaceae	≤ 10^3 - 10□ CFU/g
Escherichia coli	< 10^2 CFU/g (ideally absent)
Salmonella spp.	Absent in 25 g (0 CFU/25g)
Clostridium spp.	< 10^2 - 10^3 CFU/g
Total molds & yeasts	≤ 10^3 - 10□ CFU/g
Mycotoxins (e.g., aflatoxins)	< 20 µg/kg (ppb)

The µ value (specific growth rate) of microorganisms in a matrix such as Brewery Spent Grain (BSG) depends on various factors (including temperature, pH, nutrients, etc.) and the specific classification of the species. Under laboratory conditions, or in a high-moisture food matrix like BSG (with 82 % moisture content), and at mesophilic temperatures (25 – 30 °C), the typical values (in day^{-1}) for the microorganisms present in our BSG samples are as follows (Baranyi & Roberts (1994); Nout et al. (2007).

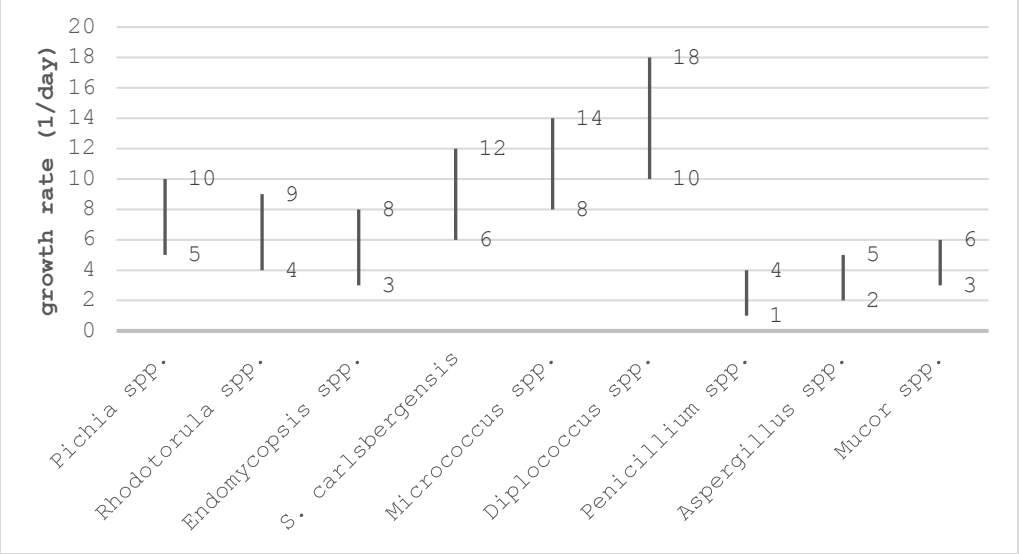

Figure 1. Specific growth rate (day^{-1}) for the microorganisms identified in BSG at 20 °C.

For filamentous microorganisms like *Penicillium* and *Aspergillus*, growth in solid substrates usually occurs slower than in liquid substrates. This is why the ranges are often on the lower part of the values. For *Pichia* (all types) as a yeast, humid conditions and nutritional values of BSG conditions might favor a faster growth, resulting in higher μ values. In the bacteria's case, the μ values might vary according to their groups (i.e., mesophilic Gram-negative bacteria grow faster than Gram-positive mesophilic bacteria) and matrix characteristics. We emphasize that these are indicative values and should be adjusted based on experimental results under specific BSG conditions (taking into consideration the nature of the substrate, processing history and the storage conditions).

Calculating degradation time. The deterioration period is calculated assuming that microorganisms follow an exponential growth model. Given an initial microbial growth of $N_0=10^3$ CFU/g and considering spoilage to occur when the load reaches 10^5 CFU/g, the exponential growth equation is applied, as follows:

$$N(t) = N_0 e^{\mu\tau}; 10^5 = 10^3 e^{\mu t};\ \mu t = \ln\left(\frac{10^5}{10^3}\right) = \ln(10^2) = \ln(100) \approx 4.605$$

$$t = \frac{4.605}{\mu}\ (days)$$

Calculating degradation time depending on temperature. Applications of the Arrhenius equation:

$$\mu = A_a\, exp\left(-\frac{E_a}{R\times T}\right) = k(T) \quad \text{and the linear form} \quad \ln(\mu) = \ln(A) - \frac{E_a}{R\cdot T}$$

Where: E_a is the activation energy; A is the pre-exponential coefficient; R=8.314 J/(mol·K)

Pre exponential coefficient (A) is calculated assuming that at 20 °C (293 K), the specific growth rate (μ) is approximately 2 day^{-1}, with activation energy

E_a = 55000 J/mol. The exponential factor is calculated:

$$\frac{E_a}{RT} = \frac{55\,000\ J/mol}{8.314\frac{J}{molK}\cdot 293\ K} \approx \frac{55\,000}{2437} \approx 22.56$$

$$e^{-22.56} \approx 1.58\ \cdot\ 10^{-10}$$

From the Arrhenius equation: $\mu = A \cdot e^{-\frac{E_a}{RT}}$; Substituting μ = 2 day^{-1}

$$A = \frac{\mu}{e^{-\frac{E_a}{RT}}} = \frac{2day^{-1}}{1.58 \cdot 10^{-10}} \approx 1.27 \cdot 10^{10} day^{-1}$$

In this example, using the Arrhenius model with typical μ and E_a values at mesophilic temperatures, the pre-exponential coefficient (A) will be approximately 1.3×10^{10} day^{-1}.

The Coefficient A is an indicator of the maximum theoretical rate of the reaction, if there was no energetic barrier. A value is experimentally determined form the linear form of the Arrhenius equation. In the above example BSG in temperature 20 °C the A value reaches approximately 1.3×10^{10} day^{-1} or approximately 1.47×10^5 s^{-1}. These values are very susceptible towards experimental conditions and might vary according to the nature of the microorganisms, nature of the substrate and other environmental parameters. High A values represent a high potential to reach maximum reaction rate, but in practice, the effective rate μ will be reduced by the energetic barrier E_a.

Below is an orientation table that represents typical activation energy (E_a) values, for some of the microorganisms. These are approximate values, expressed in (J/mol). According to the literature, these values serve as orientation and might vary depending on nutrient medium, experimental methodology and other specific growth conditions for each microorganism (Van Boekel, 1992).

Table 3: Average time (days) required the total microbial count to double.

Microorganism	**Typical E_a (J/mol)**
Pichia spp.	$50 \times 10^3 – 60 \times 10^3$
Rhodotorula spp.	$50 \times 10^3 – 60 \times 10^3$
Endomycopsis spp.	$45 \times 10^3 – 55 \times 10^3$
Saccharomyces carlsbergensis	$50 \times 10^3 – 70 \times 10^3$
Micrococcus	$60 \times 10^3 – 80 \times 10^3$
Diplococcus	$60 \times 10^3 – 80 \times 10^3$
Penicillium spp.	$40 \times 10^3 – 60 \times 10^3$

Aspergillus spp.	40 x 10^3 – 60 x 10^3
Mucor spp.	40 x 10^3 – 60 x 10^3

Microbial growth modeling. The exponential model of microbial growth was applied:

$$N = N_0 \cdot e^{\mu t} \Longrightarrow t = \frac{\ln(N) - \ln(N_0)}{\mu}$$

The Q effect was applied: for each 10 °C increase above 20 °C, the specific growth rate (μ) doubles. $\mu_T = \mu_{20} \cdot 2^{\frac{T-20}{10}}$

This is a well-known approximation, widely used in microbiology to model microbial growth as a function of temperature.

Modeling the pH-velocity (rate) dependence. One of the most widely applied models is:

$$k(pH) = k_{opt} \cdot \left(1 - \left|\frac{ph - pH_{opt}}{pH_{max} - pH_{min}}\right|^n\right)$$

Where: **k(pH)** is the effective rate at a specific pH; $\mathbf{k_{opt}}$ is maximum rate at optimal pH; $\mathbf{pH_{opt}}$ is the optimal pH for each microorganism; $\mathbf{pH_{max}}$ and $\mathbf{pH_{min}}$ are the limit values; **n** is the sensitivity factor (usually 1–2).

Let's take as an example *Pichia spp.* with these values: $k_{opt} = 20\ day^{-1}$; $pH_{opt} = 5.0$; $pH_{min} = 3.0$, $pH_{max} = 7.0$. At pH = 4.0, with n=2

$$k(4.0) = 20 \cdot \left(1 - \left|\frac{4.0 - 5.0}{7.0 - 3.0}\right|^2\right) = 20 \cdot \left(1 - \left(\frac{1}{4}\right)^2\right) = 20 \cdot (1 - 0.0625) = 18.75$$

Therefore: $k_{opt} = 20$ (at pH 5.0); $k_{effect} = 18.75$ (at pH 4.0)

In environments of optimal pH (around 6.0), enzymatic activity and microbial growth are at maximum, leading to a faster BSG deterioration. In environments of acid or alkaline pH (i.e., 4.5 or 7.5): The activity decreases and the period in which BSG remains stable increases, because deterioration happens slower. In the food industry, pH is often monitored to enhance product stability. For BSG, methods like acidification (i.e., by lactic fermentation) might lower the pH and slow deterioration. pH is a critical factor determining the deterioration kinetics and by changing the pH from an optimal level, we can manipulate the microbiological sustainability of BSG.

Influence of water activity on deterioration kinetics. In practical terms, for different a_w values (i.e., 0.4, 0.5, 0.6, 0.7, 0.8), researchers conduct experiments to measure rates such as microbiological growth, under varying water activity conditions. From these data, a function or fitted curve is graphed to represent the effect of a_w on the rate. Several approaches are applied to model this effect. For example, in the interval from the minimum value of a_w, to the optimum, a linear relationship can be used: $f(a_w) = \frac{a_w - a_{w,min}}{1 - a_{w,min}}$

Where $a_{w,opt}$ is the optimal value (usually 1.00) and $a_{w,min}$ is the minimal water activity that permits growth (i.e., 0.60).

Optimization to prolong stability. To decrease the degradation rate and achieve a stability of 2 months (60 days), we can use a strategy that combines different factors:

- Refrigerator Storage: At a temperature of 4 °C ⇒ f(T) ≈ 0.35
- BSG acidification at pH 4.5 ⇒ f(pH) ≈ 0.3
- Reducing moisture content, to decrease water activity. If $a_w \approx 0.85$, can be achieved.

$$f(a_w) = \frac{0.85 - 0.60}{0.40} = \frac{0.25}{0.40} \approx 0.625$$

$\mu = k(T) = f(T) \times f(pH) \times f(a_w) = 0.35 \times 0.3 \times 0.625 = 0.0656\ day^{-1}$

So, with these values Deterioration period will be: $t = \frac{4.605}{0.0656} \approx 70.2\ days$

This is slightly over 2 months and if we can achieve further optimization (i.e., by further reducing a_w or by using a stronger acid), the required stability can be achieved.

Results and discussions

The first step in this experimental work was the microbiological analysis of BSG. The image and accompanying table present the microorganisms identified in fresh BSG samples. The images below in figure 2 (a) & (b), show the progression of microbial colonies on Petri dishes using two different mediums: **Wort Agar (WA)** and **Plate Count Agar (PCA)**, both diluted 1:100.

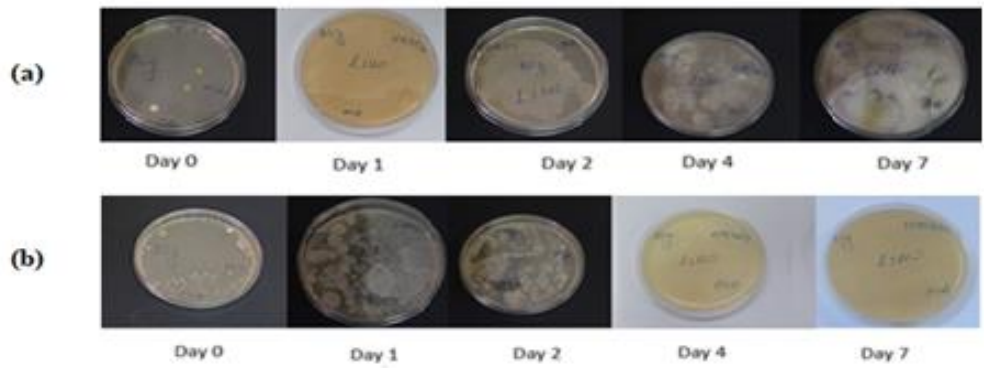

Figure 2: (a) Progress of microbial colonies in Wort – Agar diluted 1:100

(b) Progress of microbial colonies in Plate Count Agar diluted 1:100

The results of all cultivations, including both dilutions in both mediums, are summarized below in Table 4.

Table 4. Microorganisms periodically identified in BSG on Plate Count Agar (PCA) and Wort Agar (WA) during one week of storage. In dilutions of 1:10 and 1:100 for both mediums.

medium / period	PCA (1:10)	PCA (1:100)	WA (1:10)	WA (1:100)
0 days (fresh BSG)	*Pichia spp.* & *Rhodotorula spp.*	*Pichia spp.* & *Endomycopsis spp.* & *Saccharomyces cereviseae*	*Pichia spp.* (wild foreign yeast)	*Rhodotorula spp.*
1 day	Bacteria (*micrococcus* & *diplococcus*)	Bacteria (*micrococcus* & *diplococcus*)	Bacteria (*micrococcus* & *diplococcus*)	Bacteria (*micrococcus* & *diplococcus*)
2 days	Bacteria (*micrococcus* & *diplococcus*)	Bacteria (*micrococcus* & *diplococcus*)	Bacteria (*micrococcus* & *diplococcus*)	Bacteria (*micrococcus* & *diplococcus*)

4 days	*Pichia spp.*	*Rhodotorula spp.* & *Endomycopsis* & *Pichia spp.* (typical yeast)	*Penicillium spp.*	*Aspergillus spp.* & *Pichia spp.*
7 days	Bacteria (*micrococcus* & *diplococcus*)	*Rhodotorula spp.* & *Endomycopsis spp.* & *Pichia spp.* (typical yeast)	*Mucor spp.* (with highly developed aerial hyphae)	*Mucor* spp. (with highly developed aerial hyphae)

Table 5: The time (in days) required for the growth of each microorganism at specific temperatures.

Microorganism	**4 °C (days)**	**18 °C (days)**	**28 °C (days)**	**38 °C (days)**
Pichia spp.	14.0	2.3	1.4	2.3
Rhodotorula spp.	14.0	2.3	1.4	2.3
Endomycopsis spp.	17.3	2.8	1.7	2.8
Saccharomyces carlsbergensis	14.0	1.4	0.9	1.4
Micrococcus	7.0	1.4	1.0	1.2
Diplococcus	7.0	1.4	1.0	1.2
Penicillium spp.	34.7	4.6	2.3	3.5
Aspergillus spp.	34.7	3.5	1.7	2.3
Mucor spp.	34.7	4.6	2.3	3.5

The exponential growth model is used to predict the time required for microorganisms to reach the spoilage threshold. It helps in evaluating BSG storage conditions and management strategies. At 4 °C, the metabolic activity is reduced significantly, resulting in a very long growth time, ranging from about 14 to 34 – 35 days, depending on the microorganism. Under the mesophilic temperature of 18 °C, microorganisms begin to grow rapidly. For

instance, the growth time for *Pichia spp.* is approximately 2.3 days, while for slower-growing organisms like *Penicillium spp.*, it is about 4.6 days.

At 28 °C, which often represents the optimal growth temperature, doubling times decrease for most microorganisms, such as *Saccharomyces carlsbergensis* (0.9 days) and *Micrococcus* (1.0 day). However, at 38 °C, some microorganisms may experience thermal stress, leading to slightly longer doubling times compared to the optimum, e.g., *Pichia spp.* returns to about 2.3 days, while for *Penicillium spp.*, it increases to around 3.5 days.

The figure below represents the calculations for each microorganism and the decay time for each microorganism is predicted. This model shows approximate values, which depend significantly on the growth conditions (temperature, water activity, pH, etc.) and may vary depending on the specific environment.

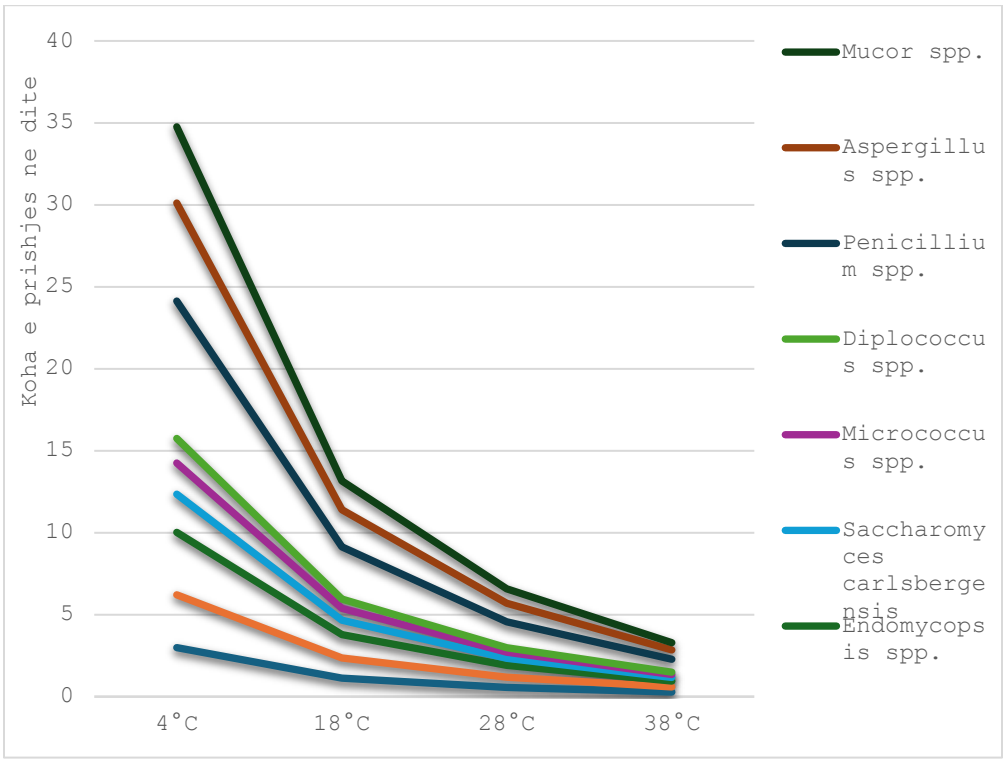

Figure 3. Prediction of decay for each microorganism.

These calculations was based on a simple exponential model that does not account for factors such as microbial competition, enzyme inactivation, or

changes in the microenvironment, all of which could modify these values. However, the graph above provides an approximate representation of the degradation time of BSG under the specified environmental conditions (20 °C and 82 % humidity).

The activation energy (Ea) values for yeasts such as *Pichia*, *Rhodotorula*, *Saccharomyces carlsbergensis*, and *Endomycopsis* fall within an intermediate range. Bacteria like *Micrococcus* and *Diplococcus* tend to have slightly higher E_a values, indicating that their growth is more sensitive to temperature fluctuations. Molds such as *Penicillium*, *Aspergillus*, and *Mucor* often exhibit lower activation energy values, reflecting their slower growth and greater adaptability to conditions that differ from those of yeasts and bacteria. These values provide a reference framework for predicting the effects of temperature on microbiological growth rates and consequently, on the microbiological stability of BSG. Exact values can be determined through specific experiments and statistical analysis of Arrhenius data.

Table 6. Effects of temperature on microbiological growth rates constants E_a and pre-exponential factor A.

Microorganism	Ea (J/mol)	A (day^{-1})
Pichia spp.	49.56×10^3	$4.93\ 10^9$
Rhodotorula spp.	49.56×10^3	$4.58\ 10^9$
Endomycopsis spp.	49.56×10^3	$3.87\ 10^9$
Saccharomyces carlsbergensis	49.56×10^3	$6.34\ 10^9$
Micrococcus spp.	49.56×10^3	$7.75\ 10^9$
Diplococcus spp.	49.56×10^3	$9.86\ 10^9$
Penicillium spp.	49.56×10^3	$1.76\ 10^9$
Aspergillus spp.	49.56×10^3	$2.47\ 10^9$
Mucor spp.	49.56×10^3	$3.17\ 10^9$

Below is a figure, that shows typical k (T) values, for the microorganisms at different temperatures (4, 18, 28 and 38 °C)

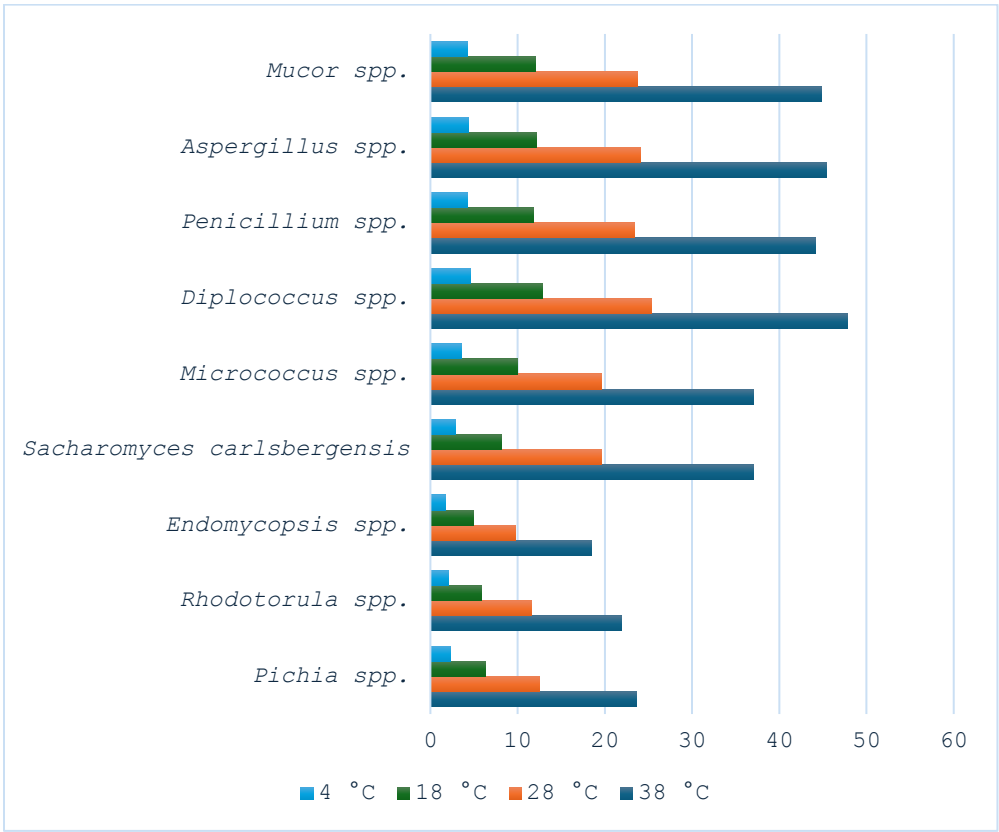

Figure 4. Typical k (T) values, at different temperatures, for each microorganism.

At 4 °C, metabolic activity is minimal, resulting in a low k (T) (also known as μ). At 18 °C, favorable conditions for mesophilic microorganisms lead to accelerated growth. The optimal growth temperature for most microorganisms is 28 °C, where k (T) reaches its peak. However, at 38 °C, k (T) begins to decline compared to its optimal value.

When discussing the effects of pH on deterioration kinetics, it is important to emphasize that pH directly affects both enzymatic activity and microbial growth. In a food matrix like BSG, pH plays a crucial role in determining which microorganisms will thrive, their metabolic rate, and their ability to degrade the material. The table below presents k (T; pH) values at different pH levels, illustrating the effects of pH on microbial growth. This data integrates the effects of both temperature and pH, providing a more comprehensive understanding of microbial activity under varying conditions.

Table 7. The effects of pH in microbial growth.

Microorganism	Temp	pH 4 f(ph)=0.2	pH 5 f(ph)=0.5	pH6 f(ph)=0.8	pH 7 f(ph)=1.0	pH 8 f(ph)=0.8	pH 9 f(ph)=0.5
Pichia spp.	4 °C	0.45	1.125	1.8	2.25	1.8	1.125
	18 °C	1.266	3.165	5.064	6.33	5.064	3.165
	28 °C	2.496	6.24	9.984	12.4	9.984	6.24
	38 °C	4.716	11.79	18.86	23.5	18.86	11.79
Rhodotorula spp.	4 °C	0.418	1.045	1.672	2.09	1.672	1.045
	18 °C	1.176	2.94	4.704	5.88	4.704	2.94
	28 °C	2.32	5.8	9.28	11.6	9.28	5.8
	38 °C	4.382	10.955	17.52	21.9	17.52	10.95
Endomycopsis spp.	4 °C	0.354	0.885	1.416	1.77	1.416	0.885
	18 °C	0.994	2.485	3.976	4.97	3.976	2.485
	28 °C	1.96	4.9	7.84	9.8	7.84	4.9
	38 °C	3.702	9.255	14.8	18.5	14.8	9.255
Saccharomyces carlsbergensis	4 °C	0.578	1.445	2.312	2.89	2.312	1.445
	18 °C	1.626	4.065	6.504	8.13	6.504	4.065
	28 °C	3.21	8.025	12.84	16.05	12.84	8.025
	38 °C	6.066	15.165	24.264	30.33	24.264	15.165
Micrococcus spp.	4 °C	0.706	1.765	2.824	3.53	2.824	1.765
	18 °C	1.988	4.97	7.952	9.94	7.952	4.97
	28 °C	3.924	9.81	15.696	19.62	15.696	9.81
	38 °C	7.414	18.535	29.656	37.07	29.656	18.535
Diplococcus spp.	4 °C	0.912	2.28	3.648	4.56	3.648	2.28
	18 °C	2.566	6.415	10.264	12.83	10.264	6.415
	28 °C	5.064	12.66	20.256	25.32	20.256	12.66
	38 °C	9.568	23.92	38.272	47.84	38.272	23.92
Penicillium spp.	4 °C	0.842	2.105	3.368	4.21	3.368	2.105
	18 °C	2.368	5.92	9.472	11.84	9.472	5.92
	28 °C	4.674	11.685	18.696	23.37	18.696	11.685
	38 °C	8.83	22.075	35.32	44.15	35.32	22.075
Aspergillus spp.	4 °C	0.866	2.165	3.464	4.33	3.464	2.165
	18 °C	2.438	6.095	9.752	12.19	9.752	6.095
	28 °C	4.81	12.025	19.24	24.05	19.24	12.025
	38 °C	9.088	22.72	36.352	45.44	36.352	22.72
Mucor spp.	4 °C	0.856	2.14	3.424	4.28	3.424	2.14
	18 °C	2.406	6.015	9.624	12.03	9.624	6.015
	28 °C	4.75	11.875	19	23.75	19	11.875
	38 °C	8.974	22.435	35.896	44.87	35.896	22.435

Each microorganism or enzyme has an optimal pH, typically between 5 and 7 for many contaminating bacteria and yeasts. At this rate, the enzymatic activity and microbial growth are at peak, resulting in a high deterioration rate. When pH is far from the optimal value (be it in the acid or alkaline direction), the enzymatic activity and microbial growth will be reduced. This way, deterioration will slow down, because the effective rate constant k_{effect} will decrease.

The combination of the three factors: temperature, pH and a_w in the degradation dynamics of BSG.

The following model represents an equation for how temperature, pH and moisture (water activity, aw) interact to determine the rate of degradation. The general model becomes:

$$k_{effect} = k_{opt} \cdot f(T) \cdot f(pH) \cdot f(a_w)$$

where: k_{opt} is the maximum velocity constant (under ideal conditions – optimal temperature, pH, water activity); **f (T):** is the factor that describes the temperature effects (i.e., at 4 °C f (T) ≈ 0.35 and at 20 °C f(T) = 1, under mesophilic conditions at 30 °C f(T) ≥ 1); **f (pH):** is a modified function that determines the deviation effect from optimal pH; if pH diverts from the optimal value (i.e., from 6 to 4.5), f (pH) decreases (i.e., f (pH) ≈ 0.2 – 0.3). $f(a_w)$ is the factor connected to water activity.

Below is an orienting table with typical values for k_{opt} and k_{effect} for the aforementioned microorganisms (the values are measured in day^{-1} and are determined under mesophilic conditions, at 20 °C):

Below is the complete table of k (T) with rounded values for *Penicillium spp.* This approach can be extended to calculate k (T) for other microorganisms under varying environmental conditions.

Table 8. k (T) values for *Penicillium spp* depending on Temperature, pH and water activity.

Temp.	a_w f(a_w)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9
4 °C (4.21)	0.85 (0.5)	0.421	1.053	1.684	2.105	1.684	1.053
	0.90 (0.7)	0.59	1.474	2.358	2.947	2.358	1.474
	0.95 (0.9)	0.76	1.895	3.031	3.789	3.031	1.895
	1.00 (1.0)	0.842	2.105	3.368	4.21	3.368	2.105
18 °C (11.84)	0.85 (0.5)	1.184	2.96	4.736	5.92	4.736	2.96
	0.90 (0.7)	1.66	4.144	6.63	8.288	6.63	4.144
	0.95 (0.9)	2.13	5.328	8.525	10.656	8.525	5.328
	1.00 (1.0)	2.368	5.92	9.472	11.84	9.472	5.92
	0.85 (0.5)	2.337	5.843	9.348	11.685	9.348	5.843

28 °C (23.37)	0.90 (0.7)	3.272	8.18	13.087	16.359	13.087	8.18
	0.95 (0.9)	4.207	10.52	16.83	21.033	16.83	10.52
	1.00 (1.0)	4.674	11.685	18.696	23.37	18.696	11.685
38 °C (44.15)	0.85 (0.5)	4.415	11.039	17.66	22.075	17.66	11.039
	0.90 (0.7)	6.181	15.453	24.722	30.905	24.722	15.453
	0.95 (0.9)	7.947	19.867	31.788	39.735	31.788	19.867
	1.00 (1.0)	8.83	22.075	35.32	44.15	35.32	22.075

Modeling the k(T) values of *Penicillium spp.*, depending on temperature, pH, and water activity provides valuable insight into how environmental factors influence fungal growth. Such a model supports accurate prediction of spoilage or toxin production risks, contributing to improved food safety, preservation strategies, and process control. It also enhances our understanding of the *Penicillium spp.* behavior under diverse conditions. By understanding how temperature, pH, and water activity affect the rate of growth, ideal storage or processing conditions can be applied to minimize contamination.

Conclusions

Predictive models are essential for forecasting the stability of BSG under varying conditions. To build a reliable predictive model for BSG degradation under the influence of temperature, pH, and water activity (a_w), it is important to integrate several types of models. The development of a predictive model requires rigorously following several key steps:

First, microbiological analysis and determination of the microbial load in BSG are needed to identify the potential contaminating microorganisms and to assess how quickly the sample may reach the permitted stability limits.

The implementation of different growth models depends on the conditions and the microorganisms.

Implementation of secondary models for environmental factors includes:

- Temperature: Incorporate an Arrhenius-type model that relates temperature to the rate constant of degradation.

- pH: Develop or use an empirical or mechanistic model that correlates pH with the degradation rate, often considering enzyme activity or chemical stability.
- Water Activity (a_w): Use a model that quantifies how moisture availability influences microbial activity or chemical reactions, sometimes through empirical correlations.

Integration Strategy: Combining these models enables the adjustment of degradation rate constants as functions of temperature, pH, and water activity. This integrated approach facilitates reliable prediction of degradation behavior across a range of environmental conditions.

By correlating these models, it is possible to simulate the combined effects of key environmental factors influencing BSG degradation.

We recommend future work focused on validating the integrated model with experimental data under real storage and processing conditions. Expanding the model to include additional factors such as microbial interactions, nutrient availability, and oxygen levels could further improve prediction accuracy and practical application.

References

Ashbell, G., Weinberg, Z. G., Hen, Y., & Filya, I. (2002). The effects of temperature on the aerobic stability of wheat and corn silages. Journal of Industrial Microbiology & Biotechnology, 28(5), 261–263. https://doi.org/10.1038/sj/jim/7000237

Atlas, R. M. (1997). Principles of microbiology (2nd ed.). Wm. C. Brown Publishers.

Baranyi, J., & Roberts, T. A. (1994). A dynamic approach to predicting bacterial growth in food. International Journal of Food Microbiology, 23(3–4), 277–294.

Bourdoux, S., Dan, L., Rajkovic, A., Devlieghere, F., & Uyttendaele, M. (2016). Performance of drying technologies to ensure microbial safety of dried fruits and vegetables. Comprehensive Reviews in Food Science and Food Safety, 15. https://doi.org/10.1111/1541-4337.12224

Cohen, E. (1985). Statistical evaluation of the Arrhenius model and its applicability in prediction of food quality losses. Journal of Food Process Preservation, 9, 273–290.

Hermansen, C., Chong, Q. K., Ho, S., Natali, F., Weingarten, M., & Peterson, E. C. (2024). Microbiome evolution of brewer's spent grain and spent coffee ground solid sidestreams under industrial storage conditions. Applied Sciences, 14(21), 9759.

https://doi.org/10.3390/app14219759

Kitaw, G., Faji, M., & Terefe, G. (2022). Nutritional and fungal load dynamics of fresh brewers' grain stored under aerobic conditions. AMB Express, 12, 10. https://doi.org/10.1186/s13568-022-01356-3

Mussatto, S. I., Dragone, G., & Roberto, I. C. (2006). Brewers' spent grain: Generation, characteristics and potential applications.

Nout, M. J. (2007). The colonizing fungus as a food provider. In Food mycology: A multifaceted approach to fungi and food.

Pinguli, L., & Malollari, I. (2022). Biochemical process engineering (pp. 179–194). Journal of Cereal Science, 43(1), 1–14. https://doi.org/10.1016/j.jcs.2005.06.001

Pinguli, L., & Malollari, I. (2023). Some alternative strategies applied to brewery spent grains for the development of sustainable recycling solutions in the agro-food industry. ERAZ. https://doi.org/10.31410/ERAZ.2023.493

Pitt, J. I., & Hocking, A. D. (2009). Fungi and food spoilage (3rd ed.). Springer.

Terefe, G. (2022). Preservation techniques and their effect on nutritional values and microbial population of brewer's spent grain: A review. CABI Agriculture and Bioscience, 3, 51.

Van Boekel, M. A. J. S. (1992). Kinetics of microbial growth: A review of modelling approaches. Critical Reviews in Food Science and Nutrition, 31(4), 289–313.

Wang, B., Luo, Y., Myung, K. H., & Liu, J. X. (2014). Effects of storage duration and temperature on the chemical composition, microorganism density, and in vitro rumen fermentation of wet brewers grains. Asian-Australasian Journal of Animal Sciences, 27(6), 832–840. https://doi.org/10.5713/ajas.2013.13668