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Fenton reaction acceleration using maltose and ascorbic acid

The degradation of various organic dyes such as methyl red (METR), indigo carmine (INDC) and methylene blue (MEBL) was studied in the presence of the hydrogen peroxide and metal ion (Cu^{2+}, Fe^{2+}). Ascorbic acid increased degradation activity of the reaction, which was promoted by sugar (maltose) addition. High oxygen consumption was also observed in the course of the Fenton reaction in the presence of maltose. Some dyes (INDC) needed the presence of oxygen for their degradation while oxygen inhibited the degradation of others (METR). Presence of the maltose in the Fenton reaction increased oxygen consumption. Electron donor (e.g. ascorbic acid) together with the suitable electron acceptor (e.g. oxygen) and an activation mechanism is needed for the additional natural substances degradation. The activation mechanism can be based on various compounds such as transient metals, hydrogen peroxide, nitrite, natural organic compounds degradation products and other. The dye bleaching in the presence of the reducing agent is probably the result of the reversible/irreversible dye reduction and irreversible dye oxidation, which can be realised in the presence or absence of oxygen.

BC 25 Beer

(Descriptors: Beer aging, mechanisms, oxidating agents, antioxidant, Fenton reaction, dye degradation.

Deskriptoren: Bieralterung, Reaktionsmechanismen, Oxidationsmittel, Antioxidantien, Fenton Reaktion, Farbstoffabbau).

1 Introduction

The ageing of beer is considered to be associated with radical degradation of certain beer constituents. Free radicals are also responsible for producing aldehydes influencing sensory attributes of beer. Fenton reaction seems to be the main pathway of radical-formation provided that transition metal ions and hydrogen peroxide are present.

The dye degradation has been widely studied to remove various kinds of pollutants present in wastewater. Fenton reaction has been often used to degrade various dyes. Radical mechanism is supposed to take place in this reaction forming oxygen free radicals and reactive oxygen species but other procedures such as illumination by visible light can also be used. Addition of organic compounds has also been found to greatly influence the Fenton dye degradation.

Chen et al. showed, that aromatic derivates, such as hydroquinone and hydroquinone-like-compounds had a catalytic action, promoting the Fenton degradation of organic compounds in the dark or under visible light irradiation(1).

Other organic compounds act in the same way. The organic matter was decomposed by the radical initiator in the presence of the transition metal which was indicated by indigo carmine (INDC) decomposition. It is the principle of the prooxidant/antioxidant test suggested earlier (2). Maltose, ethanol and methionine addition greatly increased the rate of INDC degradation.

Reducing compounds and dissolved oxygen are the main factors influencing dye degradation. Hydrogen peroxide, needed for the Fenton reaction, can be formed in the reaction between oxygen and reducing compounds. This reaction has been observed in the presence of both strong as well as weak reducing compounds (3).

Reducing compounds such as ascorbic acid, polyphenols or sulphite are often mentioned as a class of the compounds protecting sensitive beer components from the oxidation. On the other hand transition metal/ascorbic acid has been often used for the radical generation and natural compounds oxidation. Some kinds of reducing polyphenols were proved to cause higher alcohols oxidation during beer ageing (4).

The presence of strongly reducing compounds in beer and wort has been known for many years. They can be formed in the course of kilning as well as in boiling. Amino acid compounds can support reducing compounds formation in the course of Maillard reaction. These products have also been mentioned as both beer ageing promoting and inhibiting compounds (5).

The dual and of course contradictory role of the reducing compounds is worth close examining. Organic dyes, their reversible changes sensitive to oxido-reduction potential value or irreversible changes expressing dye degradation ability might help in the solving and understanding oxido-reduction processes in the course of beer ageing.

2 Experimental procedures

2.1 Chemicals

All chemicals were purchased from Sigma Aldrich except indigo carmine (Merck) and hydrogen peroxide (Chemicke zavody Sokolov, CR). The deionised water conductivity was less than 0.2 $\mu S.cm^{-1}$.

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Table 1 Methyl red (10 mg.l^{-1}) bleaching in the presence of maltose (10 g.l^{-1}) and/or ascorbic acid (0.01%), absorbance measured 15 min after H_2O_2 (0.001%) addition

	Absorbance (520 nm)	
Test	Fe^{2+} (1 mg.l^{-1})	Cu^{2+} (0.1 mg.l^{-1})
Blank	1.205	1.529
Blank + H_2O_2	1.045	1.214
Maltose + H_2O_2	1.532	1.058
Ascorbic acid + H_2O_2	0.391	1.285
Maltose + ascorbic acid + H_2O_2	0.060	1.235

Table 2 Indigo carmine (10 mg.l^{-1}) bleaching in the presence of maltose (10 g.l^{-1}) and/or ascorbic acid (0.01%), absorbance measured 15 min after H_2O_2 (0.001%) addition

	Absorbance (610 nm)	
Test	Fe^{2+} (1 mg.l^{-1})	Cu^{2+} (0.1 mg.l^{-1})
Blank	0.452	0.451
Blank + H_2O_2	0.211	0.438
Maltose + H_2O_2	0.008	0.396
Ascorbic acid + H_2O_2	0.059	0.455
Maltose + ascorbic acid + H_2O_2	0.004	0.418

Table 3 Methylene blue (10 mg.l^{-1}) bleaching in the presence of maltose (10 g.l^{-1}) and/or ascorbic acid (0.01%), absorbance measured 15 min after H_2O_2 (0.001%) addition

	Absorbance (520 nm)	
Test	Fe^{2+} (1 mg.l^{-1})	Cu^{2+} (0.1 mg.l^{-1})
Blank	1.820	1.820
Blank + H_2O_2	1.175	1.838
Maltose + H_2O_2	1.805	1.866
Ascorbic acid + H_2O_2	0.675	0.456
Maltose + ascorbic acid + H_2O_2	0.263	0.350

Stock solutions: Methyl red sodium salt (METR, c = 1000 mg.l^{-1}), indigo carmine (INDC, c = 1000 mg.l^{-1}), methylene blue (MEBL, c = 1000 mg.l^{-1}), cupric chloride dihydrate (c = 0.0268 g.l^{-1}), ferrous chloride tetrahydrate (c = 0.356 g.l^{-1}) were prepared by dissolving of the components in deionised water. Hydrogen peroxide (33 µl, c = 30% w/w) was pipetted into deionised water (10 ml) to obtain 0.1% H_2O_2. Phosphoric acid (pH ~ 4.4) was prepared by diluting 0.8 ml of the concentrated H_3PO_4 (85% w/w) with deionised water (100 ml). Sodium nitrite solution contained $NaNO_2$ dissolved in deionised water (1500 mg $NaNO_2$.l^{-1} = 1000 mg NO_2^-.l^{-1}).

2.2 Organic dye bleaching

Appropriate samples volume (usually 0.1 – 1 ml) of organic compounds solutions tested (e.g. maltose, ascorbic acid) + 0.1 ml of H_3PO_4 (pH adjusting) were pipetted into 13 x 160 mm test tubes, refilled up to 9.7 with deionised water, 0.1 ml of the stock solution of organic dye (METR/INDC/MEBL) and metal ions solution (Fe^{2+}/Cu^{2+}) added and mixed. The freshly prepared solution of the hydrogen peroxide (0.1 ml, c = 0.1%) was added in the end and mixed thoroughly to start reaction. Final composition of the mixture was organic compound, organic dye (10 mg.l^{-1}), metal ion (0.1 mg Cu^{2+}.l^{-1}, 1.0 mg Fe^{2+}.l^{-1}), hydrogen peroxide (0.001%) and H_3PO_4 (pH ~ 4.4). Higher level of Fe in comparison to Cu was chosen because of higher Fe level in raw materials and kieselguhr expected.

The absorbance of the solution containing all components except organic compound tested and H_2O_2 was measured as the blank. The other samples were measured 15 min after H_2O_2 addition at 520 nm (METR), 610 nm (INDC) and 666 nm (MEBL) (Tab. 1 – 3).

2.3 Dissolved oxygen consumption

The tested compound was dissolved in deionised water (97 ml), 1 ml of the phosphoric acid, organic dye and metal stock solutions added, stirred at 200 rpm and dissolved oxygen concentration recorded for the background determination. Hydrogen peroxide (1 ml, c = 0.1% H_2O_2) was added to start the reaction (Fig. 1). The similar experiment was carried out without dye addition (Fig. 2).

To test the nitrite influence on the electron transport between ascorbic acid and oxygen 1 ml of the ascorbic acid and H_3PO_4 were added to the deionised water (97 ml), stirred at 200 rpm and

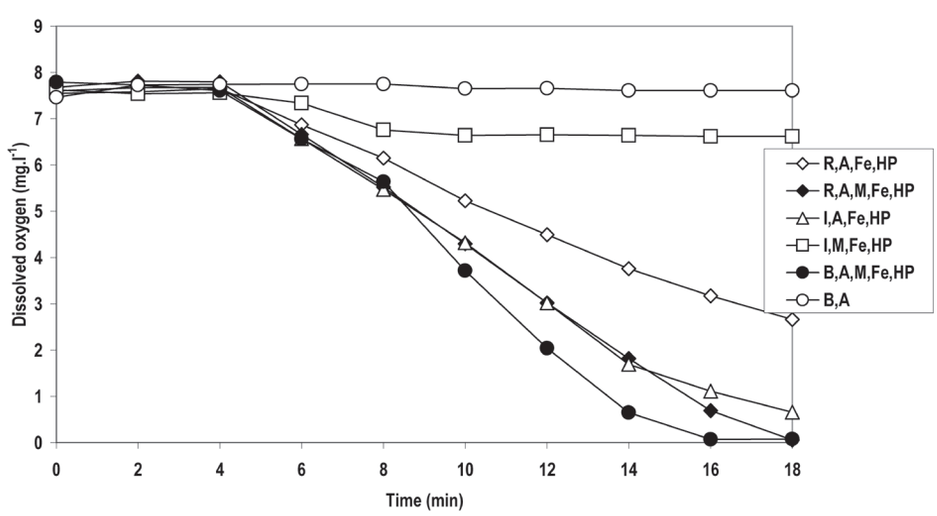

Figure 1 Dissolved oxygen consumption in asorbic acid (A, 0.01%) and/or maltose (M, 10 g.l^{-1}) solution containing organic dye (METR = R, INDC = l, MEBL = B, 10 mg.l^{-1}), Fe^{2+} (1 mg.l^{-1}) and hydrogen peroxide (HP, 0.001%) added at 4th min.

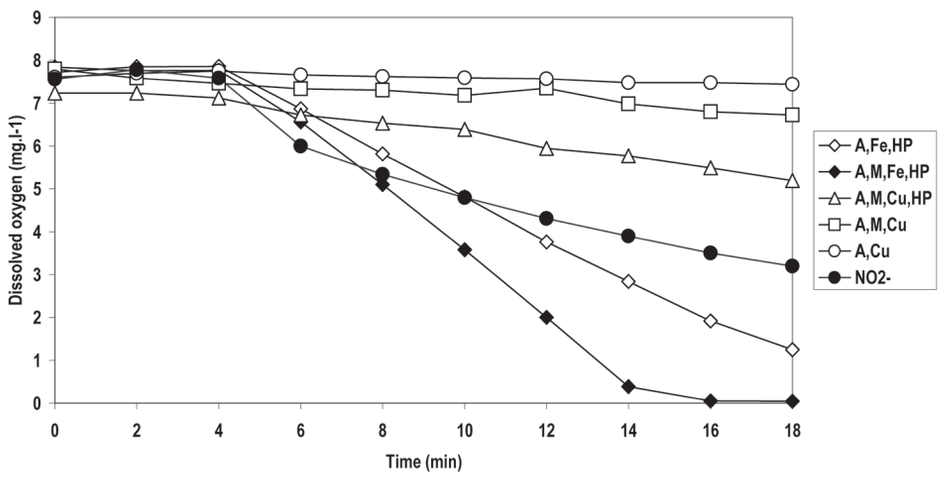

Figure 2 Dissolved oxygen consumption in ascorbic acid (A, 0.01%) solution in the presence/absence of the maltose (M, 10 g.l^{-1}), metal ion (0.1 mg Cu^{2+}.l^{-1}, 1 mg Fe^{2+}.l^{-1}), NO_2- (10 mg.l^{-1}) and hydrogen peroxide (HP, 0.001%) added at 4th min

dissolved oxygen concentration recorded for the background determination. Sodium nitrite stock solution (1 ml, c = 1500 mg.l^{-1}) was added to start the reaction (Fig. 2). Final composition of the mixture was ascorbic acid (0.01%), sodium nitrite (10 mg NO_2^-.l^{-1}) and H_3PO_4 (pH ~ 4.4).

2.4 Fenton reaction at low/high level of oxygen

Organic dye bleaching was carried out according to paragraph 2.2. Half of test tubes were shortly bubbled with nitrogen going through the needle in the stopper then evacuated and tightly sealed (low level oxygen, dissolved O_2 < 0.5 mg.l^{-1}), the other test tubes were kept under metal caps (high level oxygen, dissolved O_2 ~ 7.5 mg.l^{-1}) (Tab. 4).

2.5 Dyes mixture bleaching

Ascorbic acid solution (0.1 ml, c = 1% w/w) was pipetted into deionised water (9.5 ml) in the test tubes (13 x 160 mm), phosphoric acid (0.1 ml), organic dye (0.1 ml), metal ion solution/deionised water (0.1 ml) and hydrogen peroxide/deionised water (0.1 ml) added to get 10 ml of the final solution. Final composition of the reaction mixture was ascorbic acid (c = 0.01% w/w), organic dye (10 mg.l^{-1}), metal ion (0.1 mg Cu^{2+}.l^{-1}, 1 mg Fe^{2+}.l^{-1}) and hydrogen peroxide (0.001%), H_3PO_4 (pH ~ 4.4). Absorbance at 520 and 666 nm were measured after 240 min, the blank containing only phosphoric acid and organic dye was measured immediately after component mixing (Tab. 5).

Tabelle 4 Organic dye (10 mg.l^{-1}) bleaching in the presence of maltose (10 g.l^{-1}), ascorbic acid (0.01%), Fe^{2+} (1 mg.l^{-1}) and hydrogen peroxide (0.001%)

Dye	Bleaching time (min)	
	Dissolved O_2 < 0.5 mg.l^{-1}	Dissolved O_2 ~ 7.5 mg.l^{-1}
Methyl red	3	15
Indigo carmine	4	3
Methylene blue	15	10

Tabelle 5 Organic dye (10 mg.l^{-1}) reduction and degradation in ascorbic acid (AA, 0.01%) solution, in the presence/absence of metal ion (0.1 mg Cu^{2+}.l^{-1}, 1 mg Fe^{2+}.l^{-1}), absorbance measured 240 min after hydrogen peroxide (0.001%) addition.

Sample	Absorbance after 240 min					
	METR		MEBL		METR+MEBL	
	520 nm	666 nm	520 nm	666 nm	520 nm	666 nm
Blank (0 min)	0.930	0.013	0.117	2.00	0.850	1.96
AA	0.821	0.010	0.019	0.059	0.018	0.067
AA+Fe^{2+}	0.821	0.007	0.020	0.159	0.031	0.146
AA+Cu^{2+}	0.858	0.006	0.025	0.341	0.042	0.419
AA+H_2O_2	0.600	0.002	0.015	0.132	0.048	0.516
AA+Fe^{2+}+H_2O_2	0.192	0.000	0.060	0.327	0.259	0.502
AA+Cu^{2+}+H_2O_2	0.726	0.003	0.038	0.570	0.049	0.666

2.5 Instruments

Cadas 200 spectrophotometer (Dr. Lange, Germany) was used with 1 cm cuvette, absorbance was measured against deionised water.

Multimeter ECM – pH-O_2-µS (Dr. Lange, Germany) was used for the dissolved oxygen determination with the Clark type electrode.

3 Results and discussion

The bleaching activity of the hydrogen peroxide reacting with different organic dyes strongly depended on the kind of the dye, metal ion and organic compound added. Generally, the mixture of the hydrogen peroxide, Fe^{2+} and ascorbic acid provided high bleaching activity within all dyes.

Two different mechanisms might be involved in this reaction: Fenton reaction supported by ascorbic acid and dye reduction itself. The ascorbic is often mentioned as an agent reducing Fe^{3+} making degradation process more efficient.

The maltose addition to the Fenton reaction mixture increased bleaching activity in all cases (Tab. 1 – 3). Maltose itself inhibited or promoted the dye bleaching, which depended on the both kind of the dye and the metal ion (Tab. 1 – 3).

The course of the bleaching reaction was connected with the dissolved oxygen consumption. The highest dye degradation activity was observed with the mixture of the ascorbic acid, hydrogen peroxide and Fe^{2+} ions in the presence of maltose, which was linked to the highest oxygen consumption.

The presence of nitrite accelerated oxygen consumption in the ascorbic acid solution even without presence of the metal ions or hydrogen peroxide. The electron transfer from an acceptor to the oxygen can be probably catalysed by various compounds.

The METR degradation is an example where the absence of oxygen is needed for the irreversible degradation of the dye. Degradation reactions can probably occur under both aerobic and anaerobic condition.

METR bleaching occurred only in the mixture with the low oxygen concentration (less then 0.1 $mg.l^{-1}$). *Chapon* showed, that METR degradation was blocked in the presence of air using nitrogen flushing to increase reaction efficiency (6).

Small amount of oxygen was only consumed in the course of the INDC bleaching but oxygen was necessary for the INDC degradation (2). It has been observed, that the presence of oxygen supported INDC degradation as the absence of oxygen did in the METR solutions in beer or in reductone solutions (7).

The kind of the metal ion strongly influenced the degradation ability. The METR and INDC bleaching were often slower in the presence of Cu^{2+} than that in the presence of Fe^{2+} (Tab. 1 – 3). Maltose supported METR and INDC degradation in the Cu^{2+} presence but the ascorbic acid partially inhibited "maltose" effect. The opposite trend could be observed in the presence of the Fe^{2+}.

The ascorbic acid reduces organic dye, which is inhibited by transition metal or/and hydrogen peroxide in the course of the Fenton reaction degrading organic dye. On the other hand the rapid oxygen consumption can support organic dye reduction.

Dye bleaching processes can comprise of both reduction and oxidation even without hydrogen peroxide and transient metals presence (Tab. 5). The absorbance at two wavelengths was measured in the mixture of the ascorbic acid, metal ion and METR or MEBL to evaluate their reduction degradation. After 4 hours MEBL (but not METR) was highly reduced in the absence of the metal ions and without oxygen consumption (Tab. 5, Fig. 1). Hydrogen peroxide and metal ions partially inhibited MEBL reduction promoting METR degradation as well. MEBL could even support METR degradation in the absence of the metal ions and hydrogen peroxide.

The dye bleaching in the presence of the reducing agent is probably the result of the reversible/irreversible dye reduction and irreversible dye oxidation, which can be realised in the presence or absence of oxygen.

Organic dyes are seemed to be suitable for the degradation processes evaluation in the presence or absence of oxygen. During its reduction or activation various kinds of reactive species including oxygen free radicals, singlet oxygen or hydrogen peroxide are expected.

More common mechanisms might be taken into account comprising strong electron donor, acceptor and suitable activation mechanism taking part in the organic compound destruction. Other natural substances (e.g. sugars, aminoacids and alcohols) can be incorporated in the main electron transport promoting or inhibiting it. This mechanism is needed for the beverage anaerobic ageing explanation. The organic dye degradation might be used for the prooxidant/antioxidant studies.

4 Conclusion

- ❑ Organic dyes showed different kind of degradation behaviour in the course of the oxido-reduction reactions.
- ❑ Some dyes (INDC) degraded in the presence of the hydrogen peroxide, metal ion and sugar (maltose) other dyes (METR, MEBL) needed also reducing agent (ascorbic acid) for their degradation.
- ❑ The dyes degraded under both aerobic and anaerobic condition in the course of Fenton reaction.
- ❑ The presence of the sugar (e.g. maltose) generally promoted degradation reaction of the dyes with hydrogen peroxide, Fe^{2+} and ascorbic acid.
- ❑ Maltose degradation in the Fenton reaction was connected with the high oxygen consumption.
- ❑ The consumption of dissolved oxygen accelerated Fenton reaction catalysed by organic compound (e.g. maltose).
- ❑ Electron donor (e.g. ascorbic acid) together with the suitable electron acceptor (e.g. oxygen) and activation mechanism is needed for the additional natural substances degradation. Activation mechanism can be based on the various compounds acting such as transient metals, nitrite, natural organic compounds degradation products and other.
- ❑ The dye bleaching in the presence of the reducing agent is probably the result of the reversible/irreversible dye reduction and irreversible dye oxidation, which can be realised in the presence or absence of oxygen.

5 Zusammenfassung

Savel, J.: Beschleunigung der Fenton Reaktion durch Verwendung von Maltose und Ascorbinsäure — Monatsschrift für Brauwissenschaft 56, Nr. 1/2, 4 – 8, 2003

BC 25 Bier

Der Abbau verschiedener organischer Färbemittel, wie Methylrot (METR), Indigokarminrot (INDC) und Methylenblau (MEBL) wurde bei Vorhandensein von Hydrogenperoxid und Metallionen (Cu^{2+}, Fe^{2+}) untersucht.

Ascorbinsäure erhöhte die Abbauaktivität der Reaktion, welche durch Zugabe von Zucker (Maltose) begünstigt wurde. Bei Vorhandensein von Maltose wurde im Verlauf der Fenton Reaktion ein hoher Sauerstoffverbrauch beobachtet. Einige Färbemittel (INDC) benötigten Sauerstoff für ihren Abbau, wohingegen bei anderen (METR) Sauerstoff den Abbau hemmte. Maltose erhöhte in der Fenton Reaktion den Sauerstoffverbrauch. Ein Elektronendonor (z.B. Ascorbinsäure) zusammen mit einem geeigneten Elektronenakzeptor (z.B. Sauerstoff) und einem Aktivierungsmechanismus wird dazu benötigt, eine zusätzliche natürliche Substanz abzubauen. Der Aktivierungsmechanismus kann auf verschiedenen Verbindungen basieren, wie Übergangsmetallen, Sauerstoffperoxyd, Nitrit, natürlichen organischen Abbauprodukten und anderen. Das Bleichen des Färbemittels durch das reduzierende Agens ist wahrscheinlich das Ergebnis der reversiblen, bzw. irreversiblen Reduktion und der irreversiblen Oxidation des Färbemittels, welche durch die An- oder Abwesenheit von Sauerstoff durchgeführt werden kann.

Savel, J.: Accélération de la réaction de Fenton par l'emploi de maltose et d'acide ascorbique — Monatsschrift für Brauwissenschaft 56, No 1/2, 4 – 8, 2003

BC 25 Bière

On a examiné la dégradation de composés colorés tels que le rouge de méthyle (METR), le rouge de carmin-indigo (INDC) et le bleu de méthylène (MEBL) en présence de peroxyde d'hydrogène et d'ions métalliques (Cu^{2+}, Fe^{2+}). L'acide ascorbique augmentait l'activité de dégradation de la réaction qui était favorisée par l'addition de glucides (maltose). En présence du maltose on a observé, au cours de la réaction de Fenton, une consommation élevée en oxygène. Quelques produits colorants (INDC) nécessitent de l'oxygène pour leur dégradation, tandis que d'autres (METR) sont inhibés par l'oxygène. Le maltose augmentait dans la réaction de Fenton la consommation d'oxygène. Pour dégrader une substance naturelle additionnelle, on a besoin d'un donneur d'électrons (par exemple l'acide ascorbique) en liaison avec un accepteur d'électron souhaitable (p. ex. l'oxygène) et d'un mécanisme d'activation. Le mécanisme d'activation peut être basé sur différents composés, tels que des métaux de transition, le peroxyde d'oxygène, le nitrite, des produits de dégradation organiques naturels et autres. Le blanchiment des colorants par des agents réducteurs est probablement le résultat d'une réduction respectivement réversible et irréversible et de l'oxydation irréversible du colorant qui peut être effectué en présence ou absence d'oxygène.

6 References

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