The Blue Bottle Experiment and Pattern Formation in this System

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The methylene blue – saccharide – NaOH system, the so-called "Blue Bottle" experiment was investigated. When this system is poured into an open petri dish, spatial structures start to generate after an induction period. The induction period increases in the order of xylose < glucose < galactose < arabinose < mannose.

Introduction

The "Blue Bottle" experiment was first popularized by Campbell [1] and later by Cook et al. [2]. The experiment consists of a glass flask which is about half filled with colorless solution that turns blue when it is shaken. When left to stand, the liquid turns colorless again. This cyclic process can be repeated many times. The aqueous solution contains sodium hydroxide, glucose and methylene blue. Such an alkaline solution turns colorless upon standing, indicating that the dye has been reduced by glucose. Shaking the flask causes the blue color to return, as the reduced form of methylene blus is oxidized by atmospheric oxygen. Atmospheric oxygen is transported into the bulk solution.

$$\mathrm{CH} + \mathrm{OH}^- \leftrightarrow \mathrm{C}^- + \mathrm{H_2O}\,, \tag{1}$$

$$\mathrm{O_2\,(g)} \rightarrow \mathrm{O_2\,(soln)}\,, \tag{2}$$

$$\mathrm{O_2\,(soln)} + 4\,\mathrm{MBH} \xrightarrow{\text{fast}} 4\,\mathrm{MB}^+ + 2\,\mathrm{H_2O}\,, \tag{3}$$

$$\mathrm{MB}^+ + \mathrm{C}^- \xrightarrow{\text{slow}} \mathrm{X}^- + \mathrm{MBH}\,, \tag{4}$$

where CH is saccharide, MB^+ and MBH are the oxidized and the reduced forms of methylene blue and X^- represents the oxidation products from the saccharides.

The autooxidation of benzaldehyde illustrates an interphase transport oscillator [3] in which the transport of a gas to a liquid phase competes with the autocatalytic consumption of that gas. When that consumption becomes so rapid that the transport cannot keep up, the dissolved oxygen is depleted and a negative feedback reaction turns off the autocatalysis and permits the dissolved oxygen to be replenished by transport. A similar type of behavior was discovered by Burger and Field [4] during the oxidation of alkaline aqueous sulfide by air catalyzed by methylene blue. The catalyst and $\mathrm{O_2}$ oscillate only in a continuous flow stirred tank reactor (CSTR). Oxygen is present in relatively low concentration; it is essentially consumed in one phase of an oscillation and must be replenished by the flow for the cycle to continue [5].

We report here experimental investigation of an $\mathrm{O_2}$ – driven system, the methylene blue catalyzed oxidation of saccharides by $\mathrm{O_2}$ in basic media. We replaced the shaking of the flask by stirring of the solution and demonstrate the influence of the stirring rate and of the effect of air as a direct result of the presence of oxygen.

Nonlinear systems with many degrees of freedom can undergo nonequilibrium phase transitions characterized by a large variety of spatial or spatiotemporal patterns. Pattern formation can have different origins. Turing [6] suggested that patterns can be formed in chemical reaction – diffusion systems. These structures arise from a cooperation between molecular transport through diffusion and chemical kinetics. In addition, many other experimental systems have been described which are able to exhibit patterns, the origin of which has been discussed for many years [7]. The density changes can lead to convective motion of the solution; this itself can give rise to some interesting spatial phenomena, and chemical reactions which have suitable color changes can be used to reveal such induced convection [8], but it is separate from the diffusion-driven Turing mechanism.

Here we present spatial structures appearing in the saccharide – methylene blue – NaOH system.

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Experimental

Reagents

All chemicals were of analytical grade and were used without further purification. The solutions were prepared with doubly ion-exchanged water. A stock solution of MB^+ was made according to its nominal molecular weight, and the final concentration was determined by measuring the absorbance of diluted solution at 668 nm ($\varepsilon = 6.4 \times 10^4\ M^{-1}\ cm^{-1}$). The stock solution of MB^+ in water was kept in the dark; we never observed any measurable decomposition of MB^+ in water. All measurements were performed at $20 \pm 0.1\ °C$.

Experiments

The experiments were carried out in a thermostated cylindrical glass reaction vessel (diameter 3.5 cm, height 7.2 cm). The volume of the reaction mixture was 20 ml and the free surface area 7.98 cm^2. The reaction vessel was closed with a rubber stopper through which a commercial indication platinum macroelectrode (0.5×0.8 cm) and a reference mercury (I) sulfate electrode were inserted into the solution. In the stopper was also a hole for a capillary tube (diameter 0.4 cm in the stopper but 0.1 cm in the end) by means of which the gas was bubbled into the reaction vessel. An inert atmosphere was reached by bubbling purified nitrogen through the reaction mixture for at least 15 min, followed by a N_2 gas stream which was introduced above the reaction mixture. Nitrogen was freed from traces of oxygen by means of a solution of Cr^{2+} ions.

The potentiometric measurements were carried out using a Radelkis OH-105 polarograph. The solution was stirred magnetically with a Teflon-coated stirrer (length 2.5 cm, diameter 0.85 cm).

The reactants were added into the reaction vessel in the order: aqueous solution of NaOH, saccharide and finally solution of MB^+. Spectrophotometric experiments were carried out in a 0.5 cm spectrophotometer termostated cell (Zeiss Jena Specord UV VIS). The time dependence of the MB^+-concentration was monitored by measuring its optical absorbance, at 668 nm. The experiments consisting in mixing the reagents and pouring the mixture into a Petri dish (usually 88 mm in diameter) were performed at $20 \pm 0.5\ °C$.

Results and Discussion

a) The Methylene Blue – Saccharide – NaOH Batch System

The experimental system consists of an aqueous solution of sodium hydroxide, saccharide and methylene blue. Methylene blue monomer exists in three oxidation states [5] separated by a single electron. The three forms are referred to as MB^+, $MB^\bullet$ and MBH. The oxidized form MB^+ is colored, the reduced form MBH is colorless. In the beginning, the solution is blue. MB^+ slowly reacts with e.g. D-arabinose generating the reduced form MBH, and the potential of the platinum redox electrode decreases. Stirring of the solution causes the solution to turn blue and the potential of the Pt redox electrode abruptly increases. The rate of the bluing occurs in a few seconds. In the absence of stirring, the MB^+ form reverts to its colorless form. Mixing initiates a further bluing-debluing cycle. Typical responses of a Pt redox electrode are shown in Figure 1. The stirring rate was magnetically varied between 50 and 1000 rpm and had an effect on the reaction. A stirring rate above 300 rpm was sufficiently high to cause blue coloration which is accompanied by a change in the redox potential.

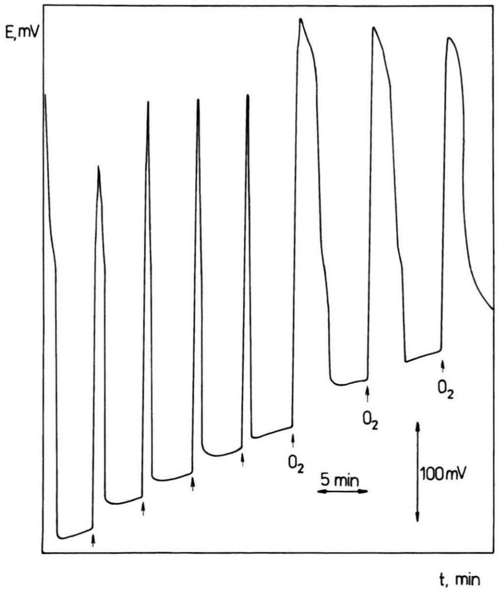

Fig. 1. Potentiometric recording of the "Blue Bottle" experiment at 20 °C. The arrows indicate the time when the stirring rate of 400 rpm is switched on and after 15 s is stopped again. Similarly, arrows indicate the beginning of bubbling of O_2 ($500\ ml \cdot min^{-1}$), which lasts 15 s. Initial concentrations: 10^{-4} M MB^+, 0.17 M D-arabinose, 0.5 M NaOH.

The behavior of a reaction mixture which is thoroughly deaerated with nitrogen is independent of the stirring rate. Neither bluing nor the changes of potential are recorded. This fact indicates that the dependence on the stirring rate is due to oxygen. The rate of transport of oxygen into the reaction mixture depends on the stirring rate. At the stirring rate 300 rpm, at which the solution turns blue, we noticed a dimple in the surface which began to increase its area. At still greater stirring rates, the dimple became a vortex which pulled air into the solution and obviously would be expected to increase the rate of transport between the phases. We used a cylindrical reactor in which the rates of rotation of the liquid are comparable at all depths. Noyes and co-workers [9] propose that the transport of molecules between gas and surface takes place in a single translational step and that the transport between surface and bulk solution is accomplished by physical mixing without significant contribution from molecular diffusion.

$$O_2\,(\text{gas}) \Leftrightarrow O_2\,(\text{surface layer}) \Leftrightarrow O_2\,(\text{bulk})$$

Interrupted bubbling of O_2 into the reaction mixture (Fig. 1) has a similar effect as interrupted stirring. The only difference is in the length of time in which the solution stays blue.

The time the solution stays blue (t) depends on the time of mixing t_{mix} (which is proportional to $[O_2]$), and under our experimental conditions this dependence is linear in the coordinates $1/t$ against $1/t_{\text{mix}}$.

The reduction of MB^+ by saccharides in the presence of dissolved O_2 is complex. Figure 2 shows the absorbance at 668 nm due to MB^+ when MB^+ is mixed with glucose (Fig. 2a) or with arabinose (Fig. 2b) as a function of time. There is an induction period (*IP*) during which $[MB^+]$ decreases only very slightly. After the *IP* the reduction of MB^+ occurs quite rapidly. We evaluated the induction period and the maximum rate of the decrease of MB^+ $v_{\text{max}} = \Delta c/\Delta t$, as the maximum slope of the rapid reaction course for glucose and arabinose (Table 1). The induction period decreases in the order of arabinose > glucose, and v_{max} decreases in the order of glucose > arabinose.

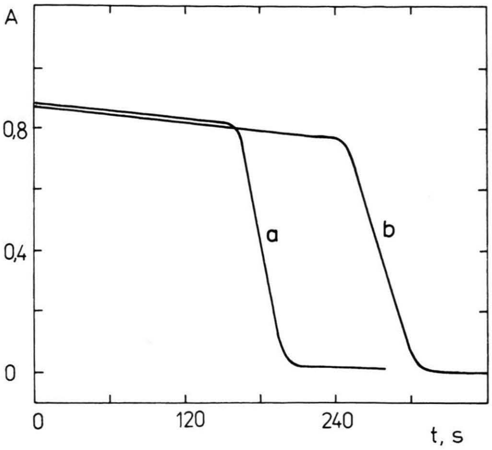

Fig. 2. Kinetics, at 20 °C of the reaction of MB^+ with D-glucose (a) and D-arabinose (b) in the presence of O_2. Initial concentrations: 2.7×10^{-5} M MB^+, 0.08 M saccharide, 0.5 M NaOH. *A* is the absorbance at 668 nm.

b) Pattern Formation in the Methylene Blue – Saccharide – NaOH System

A thoroughly mixed solution containing 0.5 M NaOH, 0.08 M saccharide and 3.4×10^{-5} M MB^+ is poured into a Petri dish (8.8 cm diameter). After a few minutes (*IP*) at 20 °C, colorless structures in the otherwise blue solution became visible in the undisturbed and uncovered solution (Figure 3). The induction period is defined as the time between the pouring of the solution into a petri dish until the beginning of the pattern formation. In the course of time a slowly changing but globally stable mosaic pattern developed. Table 2 lists several saccharides that have been observed to undergo this type of pattern formation. The *IP* increases in the order xylose < glucose < galactose < arabinose < manose, and in the same order the rates of the reactions of saccharides with MB^+ decrease in the presence of O_2. The activation energies of these reactions have been determined [2] and the order correlates also with our results (Fig. 2, Table 1).

The pattern formation was affected by the thickness l of the reaction mixture layer. The listed thicknesses are approximate because no correction was

Table 1. The induction period and the maximum rate of decrease of MB^+ for two concentrations of D-glucose and D-arabinose in a solution containing 0.5 M NaOH and 2.7×10^{-5} M MB^+. 20 °C.

	D-glucose		D-arabinose	
c/mol l^{-1}	0.08	0.17	0.08	0.17
IP/s	150	82	250	98
v_{max}/mol l^{-1} s^{-1}	7.0×10^{-7}	1.4×10^{-6}	4.0×10^{-7}	9.3×10^{-7}

Fig. 3. Pattern in a Petri dish filled to a height of 2.5 mm with a solution containing 0.5 M NaOH, 0.08 M D-glucose, 6.8×10^{-5} M MB^+, 450 s after the start of the experiment.

Fig. 4. Pattern in a Petri dish filled to a height 3.29 mm with a solution containing 0.5 M NaOH, 0.1 M D-glucose, 0.1 ml of 1% toluidine blue per 20 ml of solution, 240 s after the start of the experiment.

made for the solution adhering to the wall of the petri dish by capillary forces. The average *IP* for various depths of the solutions are given in Table 2. No patterns were observed for $l = 1.6$ mm or less. If the petri dish was protected from air with a glass cover, no spatial structures appeared in the reaction mixture. This indicates that oxygen which affects the chemical reaction and/or evaporation play a fundamental role in the pattern formation.

Table 2. The induction periods IP/s for different saccharides and for the various solution depths (l). Initial concentrations: 3.4×10^{-5} M MB^+, 0.8 M saccharides, 0.5 M NaOH. 20 °C.

Saccharides	l/mm						
	1.97	2.13	2.5	2.96	3.29	4.11	8.21
D-xylose	160			150			
D-glucose	240		230	205	175	240	235
L-glucose	250			225			
D-galactose	290			260			
D-arabinose	300			280			
L-arabinose	500	450		390	390	360	283
D-mannose	640			540			

Pattern formation may arise primarily from a chemically driven convective instability, as is exchange of matter across the air-reaction mixture interface. Because of the molecular nature of chemical systems, fluctuations in the concentrations are always present. Other physical factors might also be important to pattern formation in a chemically reacting system. Evidence that a convective instability plays a role in the pattern formation is given in Table 2. The lack of mosaic structures in reaction mixtures with depths of 1.6 mm and less was unequivocal. The convective instability is primarily a surface phenomenon. This conclusion is supported by the small dependence of the size of the "small-cell structures" on the solution of the depth. The mosaic pattern was observed also in the cases when dishes of varying shapes were used.

Also toluidine blue catalyzes the oxidation of an alkaline aqueous solution of glucose when mixed with oxygen and spatial mosaic structures are generated (Figure 4).

In summary, it is demonstrated that the "Blue Bottle" is quite rich in its dynamical behavior. In order to construct a mechanism it is important to investigate as many component reactions as possible and to develop mechanisms for each of them. The equilibrium constant of reaction (1) is 1.2×10^{-13} at 0 °C and $I = 0.03$ M [10]. The reaction (2) is discussed in [9, 11], the rate constant of the reaction (3) (direct oxidation of MBH by O_2) is determined to be $1.62 \times 10^2\ M^{-1}\ s^{-1}$ at pH 9.0 [12]. The reduction of MB^+ by saccharides in the presence and absence of O_2 as the important part of the mechanism will be the subject of future research.

Acknowledgement

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