Visualising Microbial Activity

Colorimetric Signalling Using *E. coli* with pH-Indicators and Chromogenic Substrates

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Although we are constantly interacting with the microbial world in and around us, often these interactions are not perceptible to our senses. When, for example, bacteria in our gut produce beneficial compounds that we use as a source of nutrients, these can be measured by lab techniques yet are not perceptible to the eye. For this project, a detectable sensory signal was needed to establish a foundation for communication. The available options included visual signals, olfactory signals (production of smells) or electric signals (production of a current). In the scope of *Co-Corporeality,* visual signalling was selected because of its easy detectability and the possibilities to use it as an interface for digital processing.

Bacterial selection: *E. coli*

By nature, *E. coli* is a universal and commensal member of the human gut. As a facultative anaerobic it can tolerate the presence of oxygen, growing best at 37°C and surviving at a limit of pH 3.6. Although widely understood to cause disease by the general public only a few of the known members of the *E. coli* species, such as enterohemorrhagic *E. coli* (EHEC), are pathogenic. *E. coli* is the best known of all cellular forms of life.[1] As a model organism, its structure, biochemical functions and genetics have been widely studied. This knowledge has been vital for the development of molecular biology as we know it today. *E. coli* helped to decipher the genetic code and unravel the mechanisms of DNA replication. In genetic engineering, plasmids (circular DNA) are often used with *E. coli* to study the influence of genetic modifications. Using techniques like electroporation, these plasmids can be readily inserted into the cell. For microbiologists, *E. coli* is a well-known workhorse because of its fast growth and because it can consume a wide range of usable nutrients such as glucose and lactose. Consumption of these nutrients by *E. coli* leads to metabolic products with different properties, which we will take advantage of as a method of visualising change and communication within the framework of the *Co-Corporeality* project.

Visualisation through colour change

Acidification is one of the mechanisms we make use of in the *Co-Corporeality* project for visualisation purposes. With the help of pH-indicators we visualise acidification by means of a colour change. When choosing a suitable pH-indicator it is important to consider the point at which the colour changes and also when there is a change in toxicity. Visualisation is also possible to track with nutrients: for example X-Gal, an analogue of lactose and colorimetric substrate produces a blue colour when *E. coli* mistakes it for galactose, a sugar. Alternatively, the use of neutral red allows for the detection of lactose-fermenting bacteria by turning lactose-fermenting bacterial colonies a bright red colour. We are repurposing long-established techniques from microbiology to establish a means for communication.

Methods

Chromogenic substrates • Chromogenic substrates are used in microbiology to detect the presence of certain indicator species. These substrates are mostly analogues of disaccharides and need to be enzymatically processed by the bacteria to create a colour reaction. One of best studied substrates of this kind is X-Gal (5-Brom-4-chlor-3-indoxyl-β-D-galactopyranoside). It is an analogue to lactose, a sugar that is processed by the enzyme β-galactosidase. When cleaved by β-galactosidase, it produces a blue-coloured product, thereby

1. Schaechter, M. (Ed.) (2009) Encyclopedia of Microbiology (Third Edition). Academic Press: Cambridge, MA.

allowing the visualisation of the presence of β-galactosidase activity.

pH Indicators • For many bacteria, metabolic processes result in a change of pH in the culture medium, which can be easily detected using pH-sensitive dyes. When *E. coli* utilises lactose as a carbon source, the resulting acetate and lactate lower the pH of the medium. By adding a pH-sensitive dye as an indicator in the medium, this change can be visually detected by a colour change. Since there are hundreds of indicators available, we focused on those with a colour change between pH 4 and pH 8, as this is the range in which bacteria thrive and the pH drop occurs. Another advantage of working with pH indicators is the reversibility of the reaction: the indicator is not used up in the reaction. With chromogenic substrates, the reaction is one way and irreversible.

Neutral red • Neutral red is eurhodin dye that has been used in microbiology since 1905 when MacConkey proposed its use to detect lactose-fermenting bacteria in faeces. It is particularly useful because of its pH range. In the presence of lactose-fermenting bacteria, the lowered pH results in a distinct red colour change at pH 6.8. Over the decades, MacConkey's medium has been improved by changing the pH indicator, as neutral red has been shown to have inhibitory effects on *E. coli*. Today, Bromocresol purple is used in MacConkey media.

Bromocresol purple • Bromocresol Purple (BCP) is a dye of the triphenylmethane family and used in various disciplines such as medicine or photography (see use in Fig. 1). Over the decades it has replaced Neutral Red as the pH indicator in MacConkey broth because it has less of an inhibitory effect on the growth of E.coli. At neutral or alkaline pH levels, BCP has a distinct purple colour that changes to yellow below pH 5.2.

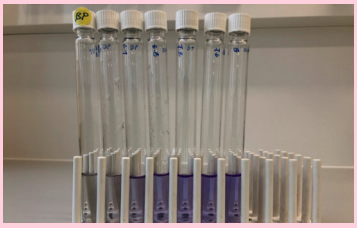

Fig. 1. Bromocresol purple at pH5.8 (left) ranging to pH8 (right).

Escherichia coli • As noted earlier, *E. coli* is one of the most studied bacteria and besides being a common gut bacteria it is widely used in microbiology and genetic engineering as a model organism. Since it possesses both β-galactosidase for cleavage of X-Gal and the ability to lower pH by metabolising lactose, it is particularly suited for this project. It is widely available and easy to grow.

Media

Liquid and solid media possess different properties and have therefore been investigated separately.

Solid media

X-Gal & MacConkey Agar • For the *E-Feed/er* project, a solid media approach was chosen. Two different media and interactions were used for the visualisation of bacterial processes. For the X-Gal reaction, standard M9 media with 15 g/L Agar was prepared. 2g of X-Gal were diluted in ~ 80mL of Dimethylformamide (DMF). The petri dishes were inoculated with *E. coli* and plates inserted into the *E-Feed/er* setup. Upon activation, drops of X-Gal solution were pipetted on the plates, resulting in a blue colour reaction after about 30 minutes.

The MacConkey Agar was prepared according to a standard recipe and inoculated with *E. coli*.

By metabolising lactose, the orange media changed to red as a result of the lowered pH. Experiments to reverse this colour reaction by addition of NaOH were not successful because the agar prevents distribution of the base within the medium.

We selected MacConkey-Agar and X-Gal as suitable media to affect the reaction of *E. coli*. When using pH-indicators in liquid media, reversibility and cycling of the colour reaction by use of sodium hydroxide (NaOH), a base, are of interest. When we interpret the colour reaction as a message that means critical environmental conditions have been reached, we can intervene and restore hospitable conditions in order to enable *E. coli* to thrive again.

The use of MacConkey-Agar or processing of X-Gal usually takes up to 48 hours and requires incubation at 37°C. Our goal was to reduce reaction time to 8 hours and more importantly, manage to get a colour change at room temperature without the use of an incubator. To process X-Gal, *E. coli* must express the enzyme β-Galactosidase. How long this sensing and expression takes is a critical factor that will be documented and adapted as necessary when determining if X-Gal is a suitable measure for visual communication between human and bacteria.

Liquid media and experimentation

1. Microfluidics & X-Gal

First experiment • For the first experiment, a Polysiloxane 3D-printed object containing the microfluidic channels was fused to a glass petri dish using plasma surface treatment. The resulting channels are leakproof and suitable for the injection of liquids (Fig. 2).

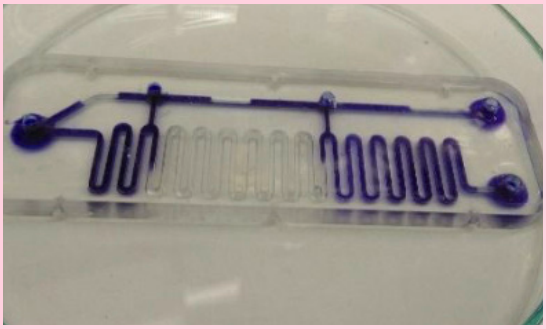

Fig. 2. Polysiloxane print fused to glass petri dish using surface plasma treatment, checking if leak proof with resazurin solution.

E. coli was grown in a shaken flask overnight in M9 media and injected into the microfluidic channels the next morning. To start the colour reaction, 100μL of 40mg/mL X-Gal in DMSO was injected into one of the channels. After 60 minutes at room temperature, no visible reaction occurred. The bacteria were transferred to a 37°C incubator. After 72h, a faint blue colouring could be detected at the point of injection (Fig. 3). The small diameter could be a limiting factor for colour intensity, so a new prototype with a larger channel diameter was prepared for the second experiment. In parallel we performed experiments with IPTG to increase reaction speed, as a 72h reaction time was not feasible in the context of an exhibition.

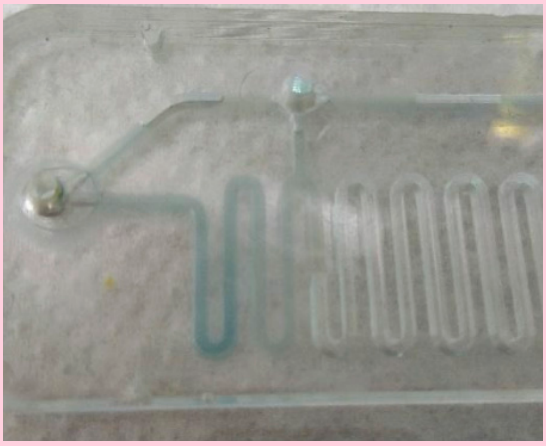

Fig. 3. Channels containing E. coli in M9 media, faint blue colour change 72h after addition of X-Gall and incubation.

Second experiment • For the second experiment, a new prototype with a larger channel diameter was used (Fig. 4). After overnight incubation of *E. coli* and injection into the channels, 100µL of X-Gal at a concentration of 40mg/mL was injected into two of the channels. Again, after 60 minutes there was no visible colour reaction and the object was transferred to a 37°C incubator. After 72h, a clearly visible colour change occurred.

Fig. 4. Colour change after 72h incubation with a larger channel diameter.

Third experiment • In genetic modification experiments, X-Gal is used to select positively modified colonies by addition of Isopropyl-ß-D-thiogalactopyranoside (IPTG). Genes introduced are controlled by a lac-operon which also contains the genetic information to produce β-galactosidase. Upon addition of IPTG as a synthetic inducer, β-Galactosidase is expressed and the addition of X-Gal leads to a blue colour reaction in those colonies that have been successfully modified.

Because of this forced β-Galactosidase expression, we speculated that induction with IPTG could lead to a faster expression of β-Galactosidase to speed up the reaction. 1 mL of *E. coli* in M9 medium was grown in Eppendorf tubes overnight and induced with 0.1mM IPTG. After incubation for 6 hours, 10 µL of 40 mg/mL X-Gal was added. After 60 minutes, no colour change was visible and the tubes were transferred to a 37°C incubator. After 24 hours a colour change was detectable. There was no substantial increase in either reaction speed or outcome.

Interestingly, one dilution series with 1–20µL of 40mg/mL X-Gal in 5mL M9 media containing *E. coli* was left in the incubator for 21 days. This series provided the strongest colour reactions, although it was impossible to tell at which time point in these 3 weeks the final intensity was reached.

2. pH Indicators

Neutral red & Bromocresol • Purple Two forms of MacConkey broth were prepared: One containing 75 mg/L of Neutral Red and one containing Bromocresol Purple at 40 mg/L. The pH of the media was adjusted to 8. An alternating series of Hungate tubes containing Neutral Red and Bromocresol Purple was incubated with 300µL of an overnight *E. coli* culture in LB-media. After 48h incubation at room temperature, a colour change was detectable.

Reversibility of colour reaction • Since in solid media the colour change could not be reversed due to the use of solid agar, we investigated if liquid media would enable us to create a cycling of colour changes. For this, 10 mL of MacConkey broth Purple was incubated with 300 µL of an overnight culture of *E. coli* grown in LB medium. As in the previous experiment, a colour change could be detected within 48 hours at room temperature (Fig. 5). Reversibility was achieved by addition of 1 mol NaOH solution in steps of 100µL until the colour changed back

from yellow to blue. Since this is a process governed by chemical interactions and not growth behaviour, the reversion occurred instantly. To investigate if *E. coli* still remains viable, three different approaches were used: Addition of lactose (10 g/L), glucose (6g/L) and LB-medium containing glucose (6 g/L). In each replicate, 10 mL of aforementioned solutions were added and flasks were again incubated at room temperature. Within 96 hours *E. coli* cultures were able to lower pH enough to produce a repeated colour change (Fig. 5). The best results were obtained from addition of LB-media with 6 g/L of glucose.

Conclusion

In the context of *Co-Corporeality,* reaction time is the most important followed by detectability. Communication by microbes could occur at a much slower speed, potentially affecting the behaviour of the bacteria through a change in the parameters of experimentation, although this would not be suitable for real-time reactions in an exhibition setting.

In solid media using X-Gal, we were able to reduce reaction time from 48 hours at 37°C to about 30 minutes at 22°C. The adaptation of temperature and time unfortunately meant the reversibility of the colour reaction was not possible for either the X-Gal or the pH-indicator experiments.

In liquid media combined with pH-indicators, only a reduction of around 6 hours was achieved at room temperature and the X-Gal experiments showed a very slow reaction time.

Using the colour change as a visual signal, a next step in visualising communication could be the implementation of a genetic reporter that would allow *E. coli* to send a visual signal (e.g. expression of a green fluorescent protein) directly to indicate that its environment was being acidified and needs to be re-equilibrated.

Fig. 5. Incubation of MacConkey broth with E. coli. Repeated colour change after 96h incubation.

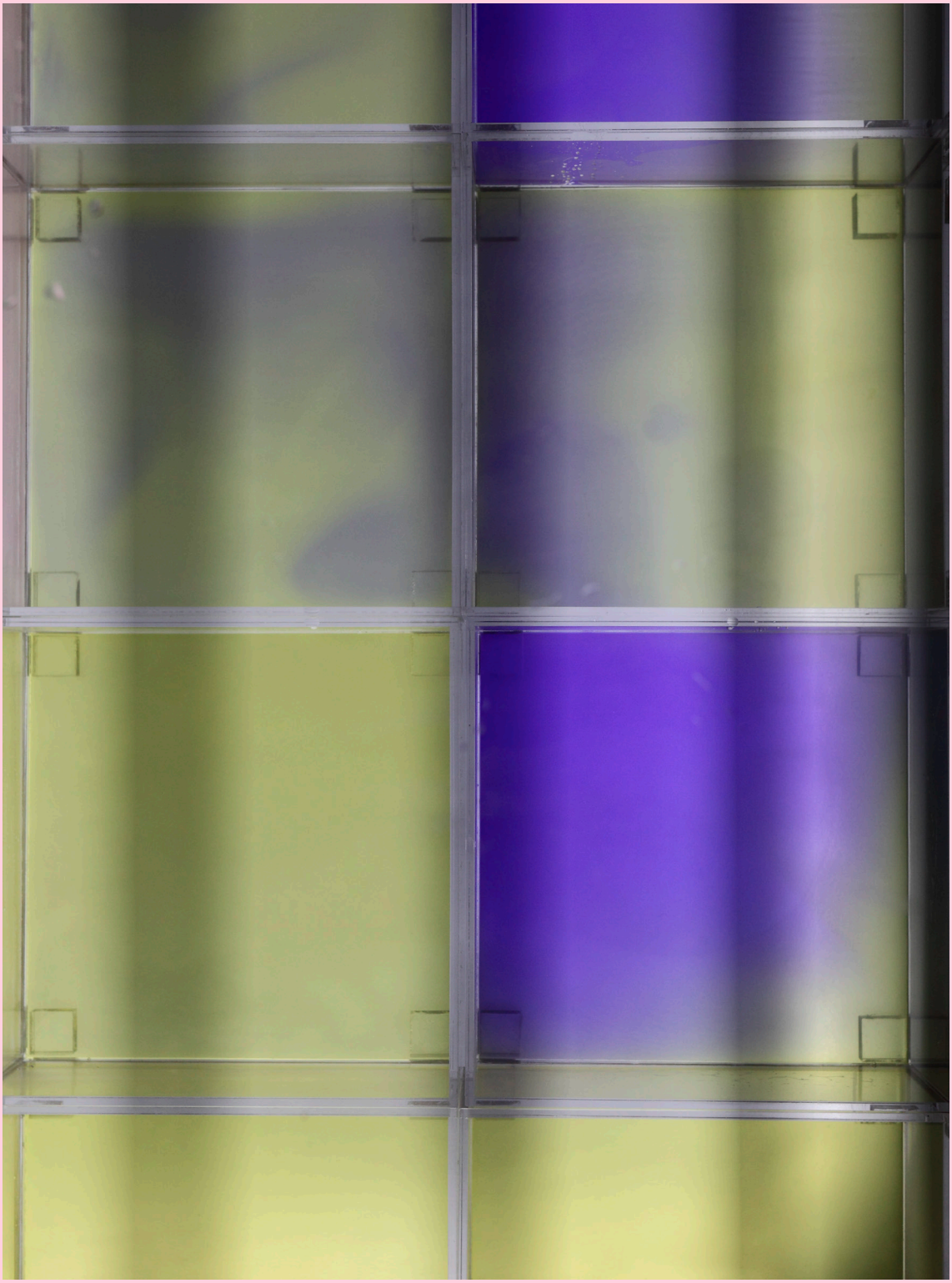

Fig. 6. E. coli in different growth stages, as shown by change of pH indicator from purple to yellow. Photo © Zita Oberwalder.