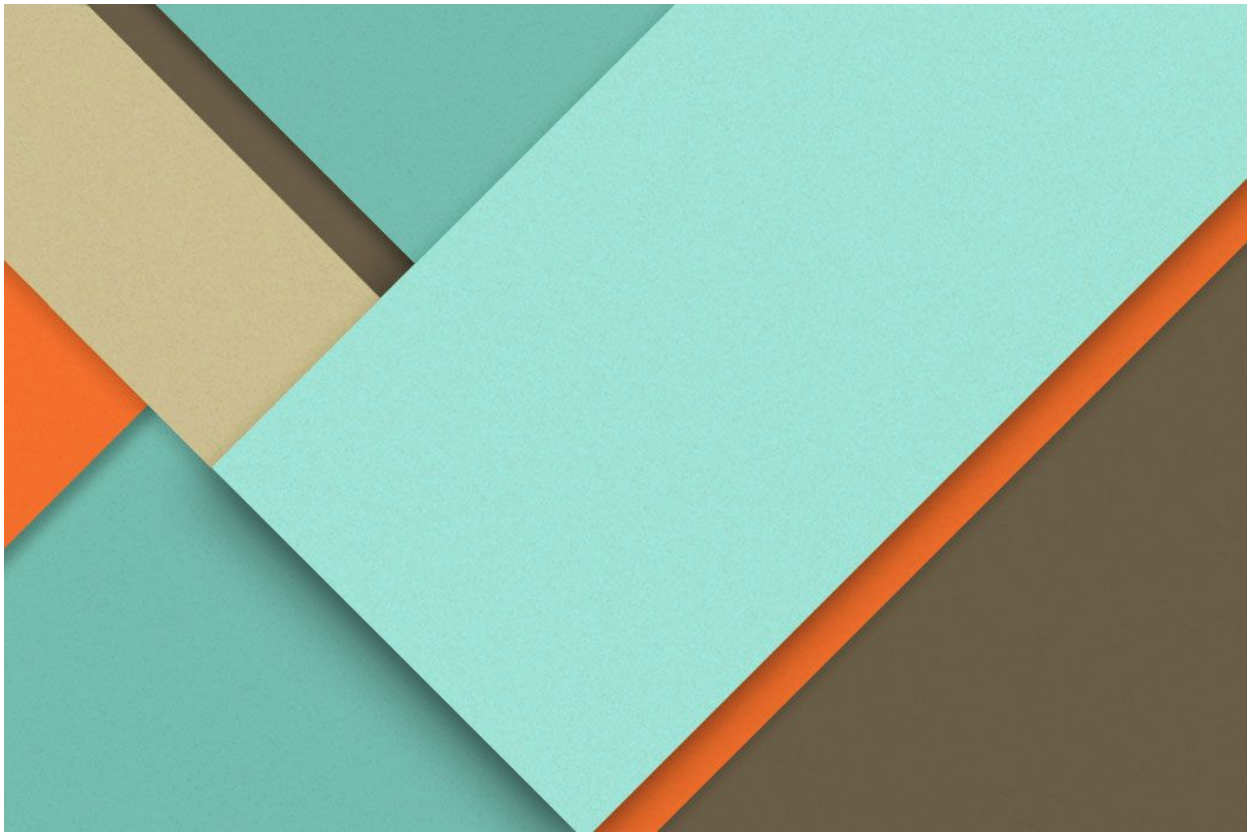


The Synthetic Biology and Chemistry of ‘Fatbergs’

Introduction

My project entailed shadowing the University of Warwick's iGEM team. iGEM is an independent, non-profit organization dedicated to the advancement of synthetic biology. The competition every year involves teams made up of primarily university students that come together to form multidisciplinary teams - the team at Warwick was comprised of Maths, Biology, Chemistry, and even English undergraduates. They work together to design, build, test, and measure their own design for a solution to a local issue.

The 2019 Warwick iGEM team are investigating the emergence of a phenomenon called a 'fatberg', which is a congealed mass of fat, oil and non biodegradable wet-wipes that has collected in sewage systems. This is a topic I previously knew nothing about and so it required a lot of research on my part to be able to integrate into the team's work.

To understand the fundamentals of the work that I completed on the placement, I had to be taught about synthetic biology by the undergrads, which was fun as I do not do Biology A-Level and I was learning something new. I learnt about PCR, genetically modifying bacteria through primers and restriction enzymes. I also used my preexisting knowledge of organic chemistry to understand the mechanisms through which fatbergs formed and broke down. I read one paper that contributed to the study of fatbergs.

ABSTRACT

The subject of interest in this report are compounds called 'Fatbergs', which are the by product of fats, oils and greases being deposited down the sink into the sewers. They block the sewers and cost millions of pounds to remove, making it incredibly cost inefficient for councils.

In my report and project, I am looking to make detailed observations about the issues fatbergs cause, and analyse the solution that the team come up with to reduce them. This will involve me using my knowledge of Chemistry A - Level, and it also requires me to learn some Biology (what I have not done since GCSE) to understand the synthetic processes being used to form and break down the fatbergs. Such an interdisciplinary project topic allows me to expand on previous knowledge and gain knowledge I previously did not have.

The formation of a fatberg:

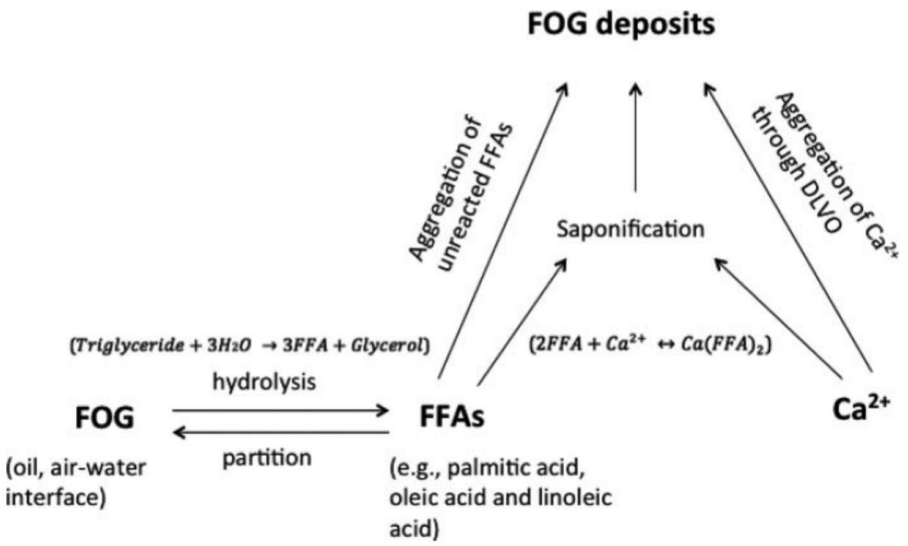


Figure 2. General understanding of FOG deposit formation in sewer pipelines (adapted from He et al., 2013).

- FOG hydrolysis occurs due to oils and grease coming into contact with water as they go down the sink, producing free fatty acids. They are discharged into sewer pipelines, and they would partition into an oily layer and flow on the wastewater surface.

- In the presence of calcium from the surrounding concrete in sewers, the saponification process (turning into soap) can occur at a fast rate and the saponified solid acts as a primary attachment point to the sewer pipe wall. The unreacted FFAs would then tend to accumulate around the core and draw additional calcium and other cations toward the solid. Different sources of calcium and fat in sewer systems also produced different colour and texture of FOG deposits.
- Solids like flushable wipes and plastic tampon applicators make up the bulk as the saponification occurs around it once it has been flushed down the toilet, making the fatbergs truly large and immense.
- So, in summary the 2 things that catalyse the formation of fatbergs - disposal of non biodegradable items like flushable wipes, and depositing oil down the sink.

METHODOLOGY TO BREAK DOWN THE FATBERG

There are 3 ways to effectively remove fatbergs, either mechanically, chemistry or biologically.

Mechanical method:

- Using hydrojets in the sewers to effectively break down
- This is not effective as it is time and energy inefficient - many fatbergs are hundreds of metres long, and so it would be an impossible task to achieve without the aid of any chemical or biological processes.
- It would also not be feasible for the iGEM solution to produce a mechanical solution as the competition focuses on synthetic biology.

Chemical method:

- Using hydrogen peroxide, $H_2O_{2,}$, which will easily decompose the fatberg.
- This is extremely unsuitable for water systems and sewers, as H_2O_2 can not only breakdown the fatberg but also the sewers themselves.

The only method iGEM could focus on is

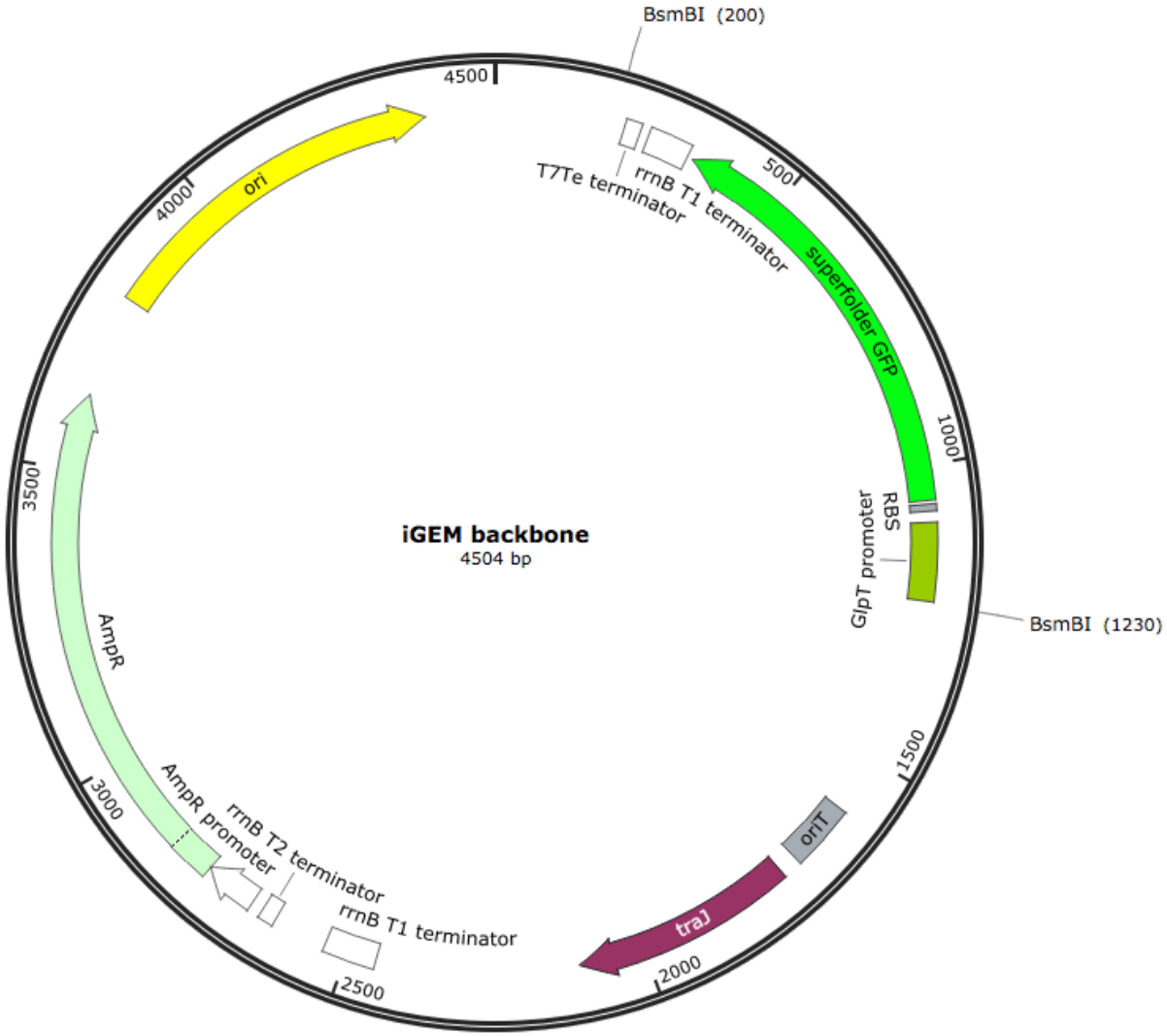
Biological Method:

- engineering bacteria (e. coli) to have an up-regulated form of the lipase gene to break down fats into triglycerides, and how this is also problematic as the

triglycerides undergo saponification under the high calcium levels of the sewers to remake a fat berg

- iGEM also tested their genetically modified plasmid in different concentrations of oils in plates.

Below is the design of the iGEM plasmid:



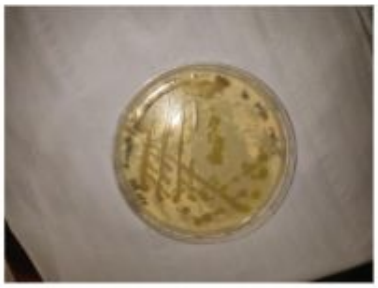
RESULTS AND DISCUSSION

The oil plate experiment:

This experiment was designed by the iGEM team to investigate the efficacy of their genetically modified bacteria - i.e look at how the E.Coli would cope in different concentrations of oil (mimicking the conditions of the sewers).

Below are pictures of the bacteria colonies that were able to grow in certain concentrations of oil:

0.5% Lipid LBA



1% Lipid LBA

5% Lipid LBA

10% Lipid LBA

I will now be discussing the results of 2 methods to characterise the soap - one carried out by iGEM, and one from a research paper about the analysis of the Cranfield fatberg.

iGEM team:

The experiments so far have been mostly unsuccessful. They believe this is due to a complication with the synthetic biology stage, i.e., they believe that the plasmid, which was the vector for a lipase enzyme producing gene, was not correctly modified.

This means that they have another month or so to produce results of their characterisation of the fatberg sample they have obtained from Liverpool. They then

require additional time to test whether their solution to adding this bacteria and the alcohol will prevent saponification.

Cranberg Water Fatberg Analysis:

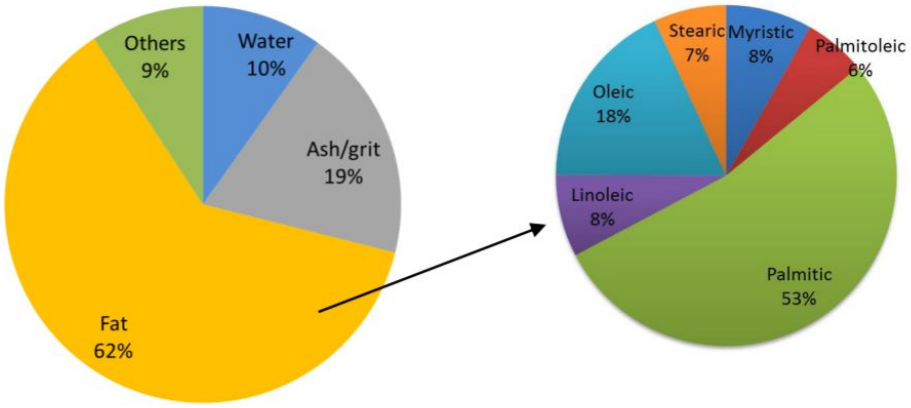


Figure 1. (a) Whitechapel sample composition and (b) Speciation of the fatty acids contained in the fat fraction of the sample.

Table 1. Fatty acids composition of some commons fats and the fatberg:

	Myristic acid C14:0	*Palmitoleic acid* C16:1	*Palmitic acid* C16:0	*Linoleic acid* C18:2	*Oleic acid* C18:1	*Stearic acid* C18:0
Sunflower oil	0.1	nd	6	62	28	3
Rapeseed oil(canola)	nd	nd	5	26	56	2
Olive oil	nd	nd	10	7	78	2
Coconut oil	16	nd	9	2	7	2
Palm oil	nd	nd	44	10	40	4
Beef fat	3	11	27	2	48	7
Butter	12	3	26	2	28	11
Chicken/turkey fat	1	6	22	20	37	6
Lard	2	4	27	11	44	11
Salmon	3	5	11	5	25	4
Fatberg	**8**	**6**	**53**	**8**	**18**	**7**

Table 2. Metal content of the fatberg sample and primary sludge metal levels from literature:

	Fatberg mg/kg (solid)	*Primary sludge mg/kg(solid)*
Sodium	116 ± 17	
Magnesium	49 ± 6	
Aluminium	293 ± 56	6442-11002
Silicon	345 ± 73	
Phosphorus	154 ± 20	
Potassium	85 ± 16	
Calcium	785 ± 307	17.8-1286
Titanium	11 ± 2	44.9-73.1
Vanadium	2 ± 0	
Chromium	4 ± 7	36.1-239
Manganese	2 ± 0	103-297
Iron	407 ± 70	4472-13847
Cobalt	0 ± 0	1.99-5.49
Nickel	0 ± 0	14.3-21.7
Copper	12 ± 3	131-256
Zinc	44 ± 5	633-997
Arsenic	0 ± 0	
Selenium	0 ± 0	
Molybdenum	0 ± 0	3.52-10.2
Cadmium	0 ± 0	1.89-6.03
Tungsten	0 ± 0	
Lead	28 ± 16	72.5-222
Mercury	1 ± 1	nd

These results are evidence of 2 things:

1. The fatbergs are formed from cooling and solidification of saturated FOG's poured down drains.
2. In addition to long chain free fatty acids, like palmitic and oleic acid, researchers have measured significant concentrations of metal ions, such as calcium in FOG deposits. He et al. (2011) further provided evidence to show that FOG deposits were similar to laboratory-based calcium-fatty acid salts, indicating that a significant portion of FOG deposits are saponification induced calcium fatty acid salts. Triglycerides were also found in trace amounts in the fatberg, suggesting that the solution to this problem is that the transport of long chain free fatty acids

and the possible release of calcium from deteriorating concrete structures needs to be reduced.

EVALUATION

The iGEM team need to reevaluate their methodology for the production of their lipase producing E.Coli bacteria - this can be done using a different DNA backbone perhaps, or different primers.

The results obtained from the oil plate experiment show that the bacteria grows well in lower concentrations of oil, and so it may not be able to withstand the conditions of the sewers. In a meeting, it was discussed that this can be improved by selecting a better vector for the gene, e.g, a different bacteria, or making the genetic engineering process more effective.

APPENDIX

1. On my first week, it was recommended to me by the undergraduate students to watch this documentary to decompose the issue surrounding fatbergs : https://www.channel4.com/programmes/fatberg-autopsy-secrets-of-the-sewers
2. Articles that are interesting and clear:

   https://microbiologysociety.org/blog/sewage-science-are-bacteria-just-the-tip-of-the-fatberg.html

   https://www.standard.co.uk/futurelondon/theplasticfreeproject/fatbergs-and-single-use-plastics-a4034686.html

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1. Cranfield Water Fatberg Analysis - A critical review of fats, oils, and greases
2. https://www.nationalgeographic.com/news/2017/08/fatbergs-fat-cities-sewers-wet-wipes-science/
3. https://www.theguardian.com/uk-news/2018/apr/24/fatberg-autopsy-reveals-growing-health-threat-londoners

BIBLIOGRAPHY

Projects that previous iGEM teams have created in relation to fatbergs:

http://2014.igem.org/Team:Sheffield/Project

http://2018.igem.org/Team:NDC-HighRiverAB/Description

Thames Water generating awareness about how to prevent fatbergs:

https://www.thameswater.co.uk/-/media/Site-Content/Thames-Water/Help-and-Advice/Helpful-literature/Accord4/Pdf1.pdf

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