Coastal biofilms

This experiment aims to observe the growth of biofilms on a substrate by exposure to seawater. We can determine whether the growth depends on the source of the seawater and whether the nature of the substrate (coated or uncoated, rough or smooth) has an effect.

Target audience: KS4

Learning outcomes

- **Biofilms are communities of microbes**
- **Biofilms are formed on a surface**
- **Biofilm formation can be inhibited**

National Curriculum links:
2006 Curriculum for KS4:
This activity covers Sc2 Life processes and living things particularly Organisms and health 'organisms are interdependent and adapted to their environments'.

Prior to 2006: KS4 Single and Double Award
This activity covers Sc2 Life processes and living things
Adaptation and competition ' how the distribution and relative abundance of organisms in habitats can be explained using ideas of interdependence, adaptation, competition and predation
Also Sc1 Scientific enquiry 'Investigative skills' (Sc1 2a-f, 2h-k, 2m-s)

CLEAPSS References
General risk assessments should be carried out prior to this investigation.

- **Activities should comply with the employer's risk assessments for fieldwork and any relevant codes of conduct. See the CLEAPSS Laboratory Handbook section 17.1 Fieldwork, but follow the employer's risk assessments where these differ.**

- **Seawater should only be obtained from sites which are unlikely to be contaminated with sewage, spilled oil or fuels or other hazardous matter. Do not sample from sites near sewage outlets or harbours, or beaches known to have 'poor-quality' water for bathing purposes. Tide times should also be checked when planning to collect the water. Good hygiene is vital though most organisms growing in seawater require a high-salinity environment and are therefore unlikely to be human pathogens.**

- **Hazardous products such as bleach and anti-fouling paints must be used with discretion and safety information on the product labels must be adhered to at all times. Alternatively consider whether these products can be substituted by other relatively low-hazard products. Consult Hazcards and if applicable Recipe Cards; the CLEAPSS Laboratory Handbook section 15.12.3 suggests various disinfectants.**

- **Health and Safety Information Document HSE 730/15 safe use of tin-free, marine anti-fouling coatings (http://www.hse.gov.uk/fod/infodocs/730_15id.pdf) states**

  The use of booster biocides in marine anti-fouling coatings is increasing. Only biocides approved under the Control of Pesticides Regulations 1986 or the Biocidal Products Regulations 2001 for use in anti-fouling coatings may be used. A recent review of booster biocides resulted in approval being granted for the continued use of 4 substances in anti-fouling coatings, some of which can cause skin sensitisation. Conditions of use established as part of the review process have to be detailed on the product labels. Users of anti-fouling coatings have to comply with these conditions including wearing the required personal protective equipment (PPE)...

Though this document applies to professionals using these products on a large scale it is also available to the public. Attention should be drawn to the fact that product labels contain important information on safety of use.

Background information

Although many bacteria can grow in a free-living, 'planktonic' state, it is quite common for them to adhere to surfaces by producing extracellular polysaccharide or in some cases by means of specialised structures termed holdfasts. The adherent bacteria produce microcolonies, leading to the development of biofilms, which initially may be composed of only one bacterial type, but frequently develop to contain several bacteria living in a complex community. In fact, almost every surface exposed to liquids and nutrients will be colonised by microorganisms.

Coastal biofilms are often seen as the slimy layer on rocks. However, sophisticated imaging techniques are required for the fine detail necessary for scientific research. One such technique is confocal scanning laser microscopy (CSLM) which allows living biofilms to be studied in more detail than conventional microscopy but without the dehydration steps of electron microscopy.

Resources needed:

- **tall buckets (about 40 cm tall)**
- **seawater collected from specific locations – see CLEAPSS reference to seawater**
- **sticks or dowels longer than bucket is wide**
- **clear, empty 2-litre fizzy drinks bottles (remove label)**
- **scissors**
- **hole puncher**
- **2 medium-size washers, fishing weights or small stones per set up**
- **2 pieces of string, each 1 metre long per set up**
- **2 litre plastic ice-cream containers**
- **magnifying glasses**
- **coating materials: e.g. Vaseline, household bleach e.g. Domestos, antifouling paint (see CLEAPSS reference for antifouling paint)**
- **small e.g.1-2cm paintbrushes**
- **newspaper**
- **sandpaper**

What To Do:

1. Collect seawater from at least three different locations (see CLEAPSS reference for seawater).
2. Fill a bucket with seawater. Label the bucket to differentiate between the different locations.
3. **Note: Take care when using sharp scissors. Be aware that the cut edges of the plastic bottle may be sharp and can be smoothed using sandpaper.** Using the scissors cut the top and bottom off the plastic bottle about 5 cm from each end. Throw away the top and bottom.
4. The remaining part of the bottle looks like a cylinder. Cut through it from top to bottom to create a rectangular piece.
5. Flatten the piece and then cut into 5 equal strips.
6. Punch a hole into each end of each strip.
7. Set one strip aside. This is the control strip.
8. Set another strip aside. With sandpaper roughen both surfaces.
9. Spread newspaper or paper towels on a table. Working over the newspaper, coat each of the other 3 strips, front and back, with a different coating using a paintbrush. Use only one coating substance for each strip. Set them on the newspaper and let them dry (drying time will vary for each coating substance used).
10. Arrange your 5 strips in a row so that they are parallel. Thread a piece of string through the hole in one end of one strip and pull it through, leaving about 7 cm hanging.
11. Tie the strip to the string. Thread the long end of the string through one hole of the next strip and tie it off about 3 cm from the first strip.
12. Continue to thread the strips onto the string, keeping them 3 cm apart. Using your second string, repeat this process on the other side of the 5 strips.
13. Tie the weights (washers, fishing weights, stones) to the 7 cm ends of the strings. Tie the other ends of your strings together.
14. Hang your setup from the stick or dowel and lay the stick across the top of the bucket so that the setup hangs down into the water.
15. All strips should be submerged, but they shouldn't be resting on the bottom.
16. Repeat for the other buckets.
17. Wash your hands. Leave the buckets and setup undisturbed for two weeks (check occasionally to make sure your top strips are still covered by water).
18. Working carefully around your setup so you don't jostle it badly, remove a few cups of water from the bucket and pour into your ice cream container placed on newspaper. Carefully move your setup to this container so that it doesn't dry out while you're working with it.
19. Touching and lifting strips very carefully so you don't rub off the biofilms, examine the surface of your strips with the magnifying glass. You'll probably see green, brown, pink or reddish spots. Rate the amount of growth on each strip on a scale of 1 to 5 (1 being the least growth). Give the uncoated strip a 3 rating and rate the other strips compared to it. Use the work sheet on the last page to record the data.
20. Create a bar graph with the different coatings labelled on the horizontal axis and the growth ratings on the vertical axis. **Wash your hands after touching the strips**.

Questions:

1. **Which coatings did the biofilms form best on? Which did they grow least well on? Vaseline usually results in good growth; antifouling paint should show the least growth.**
2. **Do biofilms grow better on rough or smooth surfaces? Biofilms will grow better on rough surfaces as there is more opportunity to adhere.**
3. **What do your results tell you about biofilm formation? Some substances can hinder the production of biofilms. We use chlorine in swimming pools to prevent colonies of algae and bacteria forming on the sides and bottom of the pool.**
4. **Can you think of some problems biofilms might cause? Biofilms build up on the bottom of boats, creating friction that reduces the boat's speed. Biofilms build up on teeth (plaque) and on contact lenses and cases.**
5. **Can you think of any useful things they might do? Biofilms break down raw sewage into less harmful substances. Biofilms can be used to grow huge colonies of microbes in vessels known as bioreactors. These can produce useful products such as enzymes and drugs.**

Alternative experiments

The experiment can be carried out using glass microscope slides instead of plastic strips. The slides can be fixed so that they slide into two document binders (plastic binders that slide over the edge of documents to avoid spiral binding or hole punching). In this way the slides can be placed next to each other to form a continuous plate between the two binders. Or the slides can be spaced at intervals. In this way the flat set up can be propped up in a bucket or placed at an angle in a waterway.

An alternative small-scale practical set up is detailed in the Revised Nuffield Biology Text 3: Living organisms and their environment (1966/1975) and its Teachers' Guide.

Extension work

This experiment could be extended to look at biofilms growing in low tidal zones. Put panels (e.g. fibre glass or plywood or steel) into seawater below the low tide point. These should get colonised over time, initially with algae and bacteria then onto seaweeds and higher animals. This creates interesting debates on what constitutes a biofilm. The set up could be revisited periodically and investigated for the different type of organism colonising the surfaces. An investigation could be made into the effect of putting in a copper plate. The metal ions might well deter certain organisms. Alternatively paint some panels with antifouling boat paint.

Useful websites

Biofilms online – educational resources on biofilms
http://www.biofilmsonline.com/cgi-bin/biofilmsonline/index.html

Abstracts from the Society of General Microbiology
http://www.socgenmicrobiol.org.uk/meetings/pdfabstracts/exeter2000abs.pdf

A Friendly Guide to Biofilm Basics
http://www.erc.montana.edu/CBEssentials-SW/bf-basics-99/bbasics-01.htm

The Microbial World: Microorganisms and microbial activities
http://helios.bto.ed.ac.uk/bto/microbes/

The Microbial World: Biofilms
http://helios.bto.ed.ac.uk/bto/microbes/biofilm.htm

Marine Science Online Magazine
http://www.virtue.uib.no/magazine/teaching/Biofilm/biolfilm.htm

Teacher resources from the Maryland Sea Grant Program
http://www.mdsg.umd.edu/Education/biofilm/

Biofilm facts
http://www.microbe.org/news/biofilms.asp

Coastal biofilms: Factors affecting growth

Date:

Name:

1. Touching and lifting strips very carefully so you don't rub off the biofilms, examine the surface of your strips with a magnifying glass. You'll probably see green, brown, pink or reddish spots.
2. Rate the amount of growth on each strip on a scale of 1 to 5 (1 being the least growth).
3. Give the uncoated strip a 3 rating and rate the other strips compared to it.
4. Create a bar graph with the different coatings labelled on the horizontal axis and the growth ratings on the vertical axis.

Wash your hands after touching the strips.

Source of water	Control	Roughened surface	Vaseline	Bleach	Antifouling paint