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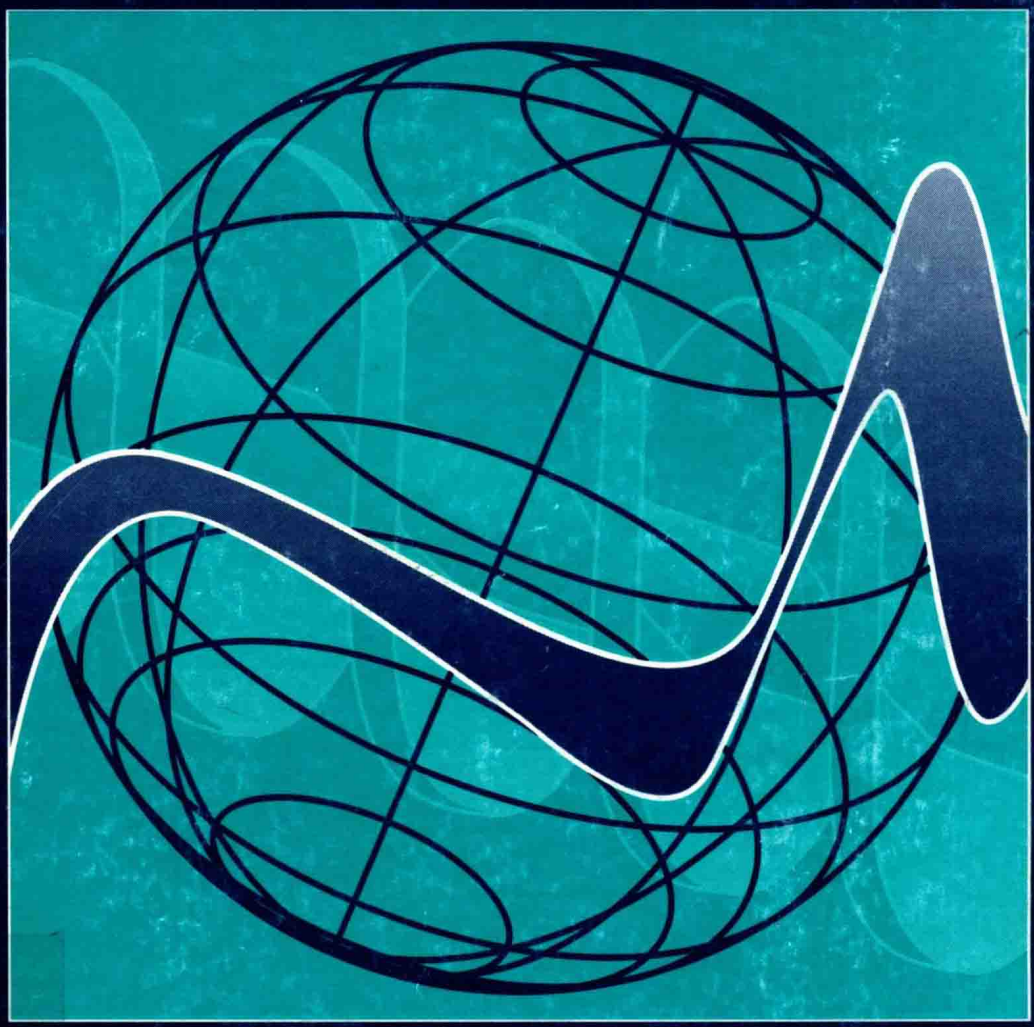

Biofilm Systems III

Editor: P Harremoës

BIOFILM SYSTEMS III

Selected Proceedings of the IAWQ 3rd International Specialised Conference on Biofilm Systems, held in Copenhagen, Denmark, 27–30 August 1996

Editor

P. HARREMOËS

Department of Environmental Science and Engineering,
Technical University Denmark, Lyngby, Denmark

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Tel: (0171) 839 8390
Fax: (0171) 839 8299, e-mail: iawq@compuserve.com

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BIOFILM SYSTEMS III

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PREFACE

Biofilm reactors have been through a century of development. Why hold another conference on the issue? The basic reason is that the design and operation of biofilm reactors have advanced from simple empirical approaches to sophisticated biotechnology and as such are under constant development.

Biofilm technology is advancing. Practical application for water purification is on the increase as an alternative to activated sludge systems. Knowledge of the performance of biofilm reactors and other biofilm systems is improving. These proceedings bring the result of another of a successful sequence of conferences on biofilm science and technology.

Basic research over the last few years has increased significantly. That is the result of fundamental research using new scientific approaches, often transferred from other disciplines.

The control of the processes in biofilms and the technology of biofilm reactor application is fit for full-scale application. Novel processes have been introduced from other disciplines.

The Programme and Organizing Committees welcome you to the results of our efforts, with hope that you will find the proceedings professionally rewarding as a stepping stone for better understanding of the processes and for better performance in practice.

I want to thank everybody who has contributed.

Poul Harremoës
Professor, Department of Environmental Science and Engineering,
Technical University of Denmark, Lyngby

BIOFILM STRUCTURE AND PROPERTIES

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BIOFILM MORPHOLOGY IN POROUS MEDIA, A STUDY WITH MICROSCOPIC AND IMAGE TECHNIQUES

John E. Paulsen*, Eirik Oppen* and Rune Bakke**

** RF-Rogaland Research, Box 2503, Ullandhaug, N-4004 Stavanger, Norway*
*** HiT-TF & Tel-Tek, Kjølnes Ring 56, N-3914 Porsgrunn, Norway*

ABSTRACT

Biofilm activity, behaviour and our ability to control biofilms depends to a large extent on mass transfer phenomena in the biofilm, at the biofilm-liquid interface and in the bulk liquid. Biofilms respond to changing mass transfer conditions by adjusting morphology, thereby optimising the exchange of matter with their surroundings. Observing biofilm morphology and mass transfer in relevant fluid dynamic conditions can therefore yield essential information to understand and model biofilm behaviour. Lack of such knowledge, as the case is with regards to biofilm behaviour in various porous media, such as sandstone reservoirs, limits our ability to predict biofilm effects. A transparent porous media replica of a sandstone reservoir with cybernetic image processing has been designed to study biofilm related transport phenomena in porous media.

The porous medium was inoculated with a mixed bacterial culture and fed a sterile nutrient solution in a once through flow mode. The biofilm was observed by microscopy with automated image analysis. This novel integrated software/hardware cybernetic design allows near real-time, essentially simultaneous, surveillance of several critical sites in the porous network and facilitates selective recording and compilation of observations as a function of the biological activity at each particular site. Biofilm biomass distribution in space and time (morphology and morphological changes) are thereby recorded at a representative selection of sites in the porous structure. Local in-pore flow velocity measurements were carried out by measuring the velocity of suspended particulate matter such as detached cells or clusters of cells. The influence of biofilm morphology on convective mass transport could thereby be observed and recorded. This effect, on a meso scale, was also monitored by sensitive, automated pressure drop measurements across the porous medium cell. Important observations so far include:

- *Bioweb*; the biofilm morphology in porous media is very different from the "classical film", as it appears more like a spider web where each strand varies in size and shape.
- The biofilm maintains a large surface area and minimal biofilm depth, thereby minimising mass transfer resistance between the fluid and the biofilm phase, under the conditions tested.
- The biofilm influences the convective flow through pores both locally within pores and effecting the flow distribution between pores. Pores with high initial permeability thereby become less permeable, diverting more flow to less permeable zones in the porous matrix. Large variations in this picture were observed, demonstrating the need for a sophisticated experimental apparatus with high sampling capacity to investigate such an intricate system.

The observed biofilm behaviour in porous media has important theoretical and practical implications. Flow diversion and permeability effects are of immediate practical importance, improving the prospects for biological treatment of reservoirs. The information obtained in this study will be applied in mathematical simulations of ground water reservoirs, bioremediation and biological enhanced oil recovery. © 1997 IAWQ. Published by Elsevier Science Ltd

KEYWORDS

Automated cybernetic monitoring; biofilm; micro model; nitrate-reducing bacteria; porous medium.

INTRODUCTION

Offshore oil production frequently involves the injection of sea water into the oil bearing strata to maintain pressure and dislodge oil. Sea water contain a large and diverse population of bacteria. As the sea water enters the formation matrix and percolates into the pores, the bacteria distribute likewise. Many of the indigenous sea water bacteria are able to sustain life despite the harsh conditions offered in such environments (Paulsen *et al.*, 1995). The injection and recycling of sea water into an oil reservoir implies growth and distribution of prokaryotic species, that eventually will affect the flow patterns, the chemistry of the liquids, and the production profiles. Although the phenomenon has been known for many years (Hart *et al.*, 1960; Kalish *et al.*, 1964; Jenneman *et al.*, 1982), the concept of sessile mode of life and structured biofilms have developed during the last 20 years, thanks largely to the pioneering work of Costerton and Characklis (Characklis and Marshall, 1990). Today it is recognised that the prevalent life form under the dynamic conditions found in water flooded oil reservoirs is the sessile mode of life in a biofilm (Costerton, 1994.). The properties of the bacteria, flow velocity of the fluid, nutritional aspects, water chemistry, physio-ecology, and so on, are factors that influence the transport, distribution, settlement, and growth of the micro-organisms (Mitchell and Nevo, 1963; Fletcher and Floodgate, 1972; Jang *et al.*, 1983; Loosdrecht *et al.*, 1990; Cunningham *et al.*, 1991). The physical properties of the formation matrix, i.e. the pore sizes and distribution, influence the entrainment and distribution of the species by size exclusion, among other factors. In a large-scale perspective, this has major implications. The complexity of the system makes it difficult to design lab experiments that truly mimic a real situation. This is also illustrated by a long distance between an injector and a producer, where gradients related to temperature, nutrients and chemistry occur. The greatest challenge in modelling such systems is probably not to mathematically describe the interrelated phenomena coherently, but rather to develop experimental systems and strategies to generate valid data to calibrate the models.

A microbial research program aiming to study microbial plugging in porous sandstone was initiated in 1992 (Skjæveland *et al.*, 1996). Currently, many oil fields in the North Sea undergoing water flooding suffer early water breakthrough due to permeability heterogeneity. For this reason water diversion techniques are attractive, and the use of biomass as a plugging agent may be feasible.

Elaborate studies on sea water bacteria have been carried out (Paulsen *et al.*, 1995) as part of the research program, where the aim was to stimulate the injected, indigenous sea water bacteria to preferentially reduce the permeability of high permeability zones. It was also essential to develop experimental systems where it was possible to observe transport phenomena and monitor biomass accumulations distributed in time and space in porous media with realistic flow conditions. For this purpose we needed an integrated software/hardware cybernetic system that allowed near real-time and semi-simultaneous observation of biological changes at several sites, in a flow cell with a realistic optically observable porous network.

A series of water flooding experiments, with and without oil in the cell, have been carried out in this experimental system while it was being developed, to test its possibilities and limitations, and to observe the behaviour of the microbial community under such conditions (unpublished). The most important observation made during these tests was that biofilm morphology changed dramatically after a while in every test, turning into what we have termed a *bioweb* with strands criss-crossing the pores. Observations of oil droplets and detached biofilm from "upstream" getting caught in the web emphasised the significance of this morphological development on mass transfer phenomena in porous media (Fig. 1.) On this basis the experiment described in this paper was designed to increase our knowledge of how, where and when biowebs develop in porous media. At this point we did not focus on the overall mass balances, but applied the cybernetics to record local relative biomass changes, how the flow pattern and pore geometry affected the growth of biofilm in the porous matrix and vice versa.

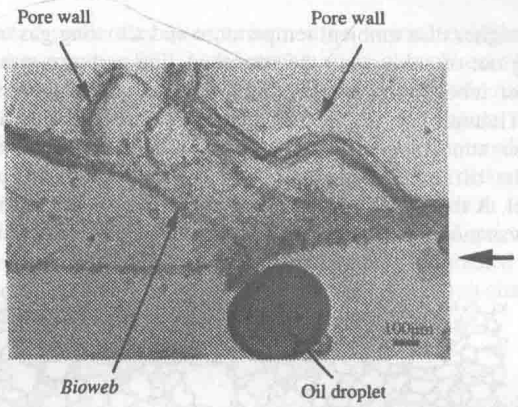

Figure 1. Digitised picture from a video film. Continuous injection of sea water and nutrients into a porous network with trapped oil droplets eventually led to the development of a bioweb. Arrow indicates the direction of fluid flow.

METHODS

A transparent 2D-glass micro model was firmly attached to a servo-controlled positioning table on an inverted microscope (Olympus IMT-2). The positioning table was interfaced with a multicontroller (Fig. 2). A CCD camera was connected to the microscope, and transferred signals to a VCR and a monitor. A computer (Macintosh Quadra 800) was interfaced with all the electronic equipment. A software program was designed to automatically control the monitoring and data acquisition.

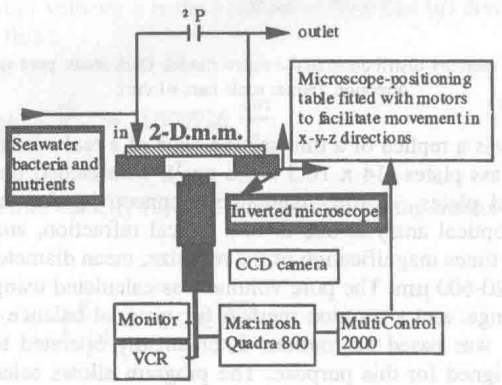

Figure 2. Schematic of experimental set-up.

A mixed population of sea water bacteria, collected in the North Sea at 50 m below sea surface, off the coast of Stavanger, was used as inoculum. The sampled water was aseptically sieved through 20 μm Millipore filter and pre-incubated 24 hours in dark, anaerobic environment before injection into micro model. The pursued process was based on nitrate-reducing bacteria; previous classification screening using Biolog MicroPlates, showed the nutrient medium selected for bacteria affiliated to the genera *Vibrio* (17%), *Acinetobacter* (8%) and *Shewanella* (33%). The residual 46% of the total population was not identified. The sea water was amended with: KNO_3 1.63 g/l, Glucose 10 g/l, and 5 ml/l of a vitamin solution (Biotin 5 mg/l, Riboflavin 10 mg/l, Thiamine 30 mg/l, Nicotinic acid 30 mg/l, Pyridoxin-HCl 50 mg/l, Ca-D Panthothenate 25 mg/l, vit. B_{12} cyanocobalamin 10 mg/l, p-aminobenzoate 10 mg/l) before injection into micro model. Fifteen pore volumes (PV) nutrient-enriched sea water was injected. The flow rate was 0.01 ml/min and PV 0.97 ml, which imply that the inoculation injection lasted 24.25 hours. Subsequently, filter-sterile sea water (0.22 μm Millipore filter) amended with the nutrients as during inoculation was injected continuously. The process was run at ambient temperature for 10 days. All injected solutions were degassed, by heating

solutions to a temperature higher than ambient temperature and allowing gas to diffuse out of solution for 48 hours to avoid gas coming out of solution in the model. A line pressure was also maintained in the micro model by fixing the outlet tube 50 cm above the flow cell. All solutions were kept in piston driven displacement cylinders (Titanium), tightly locked after degassing. Preparation of the solutions was conducted in an anaerobic atmosphere. A high precision HPLC pump (Waters 590) was driving the displacement cylinder. The differential pressure was measured by a Honeywell pressure transmitter, connected across the model. A thermal sensor (Palladium) was glued on top of the glass model. Differential pressure and temperature was monitored automatically by a Labview™ software program.

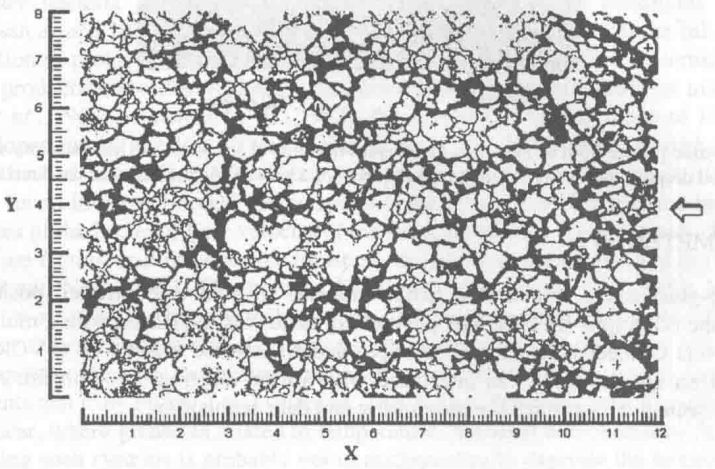

Figure 3. Digitised image of mercury distribution in the micro model. Dark areas: pore space, arrow indicates flow direction. (Note: scale bars ≠1 cm).

The micro model (Figure 3) was a replica of a thin section slice of a real core from a North Sea formation. The model consists of two glass plates (14 x 16.5 x 0.6 cm^3), with etched pattern covering 11.5 x 9 cm. Spacing between the mounted plates, ~5 μm, maintained connectivity for the whole system. The glass material allows high quality optical analysis due to low optical refraction, and may be cleaned and used repeatedly. The replica is a 10 times magnification of the real size; mean diameter of the pores ~770 μm, and the pore throats range from 120-600 μm. The pore volume was calculated using a standard chemical tracer technique with volume exchange, and a titration method for material balance calculation. Surveillance of changes in the model system was based on routines automatically operated by a software program with integrated image analysis designed for this purpose. The program allows selection of individual sites for repeated analysis, that may be changed any time during the experiment if required. During the basic procedure each individual site is monitored one by one at predetermined intervals, detecting changes by comparing the present image with the previous. Detected changes trigger the system to record, saving the image, position and time of recording, and a numeric expression of the change. The system may monitor the full-scale model or restricted areas. Restricted area monitoring implies faster collection routines and was applied in this study. The processing unit of the surveillance system was a Macintosh Quadra 800 equipped with 20 Mb RAM, 200 Mb hard disk and an eight bit grey level frame-grabber card. Images were collected from the camera monitoring the microscopic view. The microscope was fitted with different objectives, including a long-range objective (ULWD-CDPlan20PL). An on line time lapsed video cassette recorder (VCR) permitted live video recording. To optimise the observation space, the microscope-connected and the peripheral object movements were computer controlled. Automated focusing was an available option, not applied in this study. Image contrasts were enhanced by changing the view path from bright field to dark field, or interference contrast (NIC). Images were collected by repeated monitoring of 13 fixed reference positions of particular interest, e.g. some narrow and some wide pores. The interval between the optical analysis of each individual site was 2 h. Collected images were directly transferred to video tapes. A semi-

automatic scanning of 5 selected paths was also performed on several occasions, following the flow of suspended particles through these paths. Still photographs were shot whenever events of interest were discovered. Captured images (388 Kb) were saved on the hard disk to maintain maximum speed. The limited storage capacity required periodical transfer to alternative media. The computer controlled VCR facilitated single frame storage and retrieval, allowing individual transfer of the captured images, releasing storage capacity on the hard disk. Local in-flow velocity of particulate matter was determined by viewing real time video and measuring travelled distance per unit time, on the monitor. Growth of biomass was determined numerically by image analysis. Images of selected sites were collected prior to the flooding. As the experiment progressed, new images from the same sites were digitally subtracted from the reference images yielding a numerical difference as a relative measure of biomass accumulation change as a result.

RESULTS AND DISCUSSION

The question whether the flow conditions in the micro model are comparable to those in reservoirs was first analysed as follows. The micro model is two-dimensional; regarding the mean pore depth as a third dimension, the following equations apply. The mean depth of the pores was measured at 200 µm, by focus metering, analogous to optical biofilm thickness measurements (Bakke and Olsson, 1986) The total pore volume of the replica model, 950 µl, was calculated using a tracer technique. Adjusting for in- and outlet voids, PV = 0.919ml, and porosity is given by:

$$\phi = \frac{PV}{V_{total}} \Rightarrow 0.46 \tag{1}$$

The pump rate was 0.01 ml/min. The theoretical expected velocity may be calculated as described by Lake, (1989). The superficial (Darcy) velocity u is the volumetric flow rate (q) divided by the macroscopic cross-sectional area (A) normal to flow:

$$u = \frac{q}{\mathrm{A}} \Rightarrow 0.000926 \frac{cm}{s} \tag{2}$$

The interstitial velocity; the true velocity (v) of a fluid element in the matrix is calculated by incorporating the porosity:

$$v = \frac{q}{\mathrm{A} \times \phi} \Rightarrow 0.002 \frac{cm}{s} \tag{3}$$

This is nearly 173 cm per day; a realistic velocity of injection water at a near-well zone. The Darcy permeability of the micro model may also be calculated:

$$k = \frac{\mu \times q \times l}{A \times \Delta P} \tag{4}$$

Based on the recorded pressure readings (Fig. 4), the permeability is difficult to determine. The large diurnal fluctuations are explained by temperature changes from day to night time. During day time the temperature varied 24-29°C, dropping to 17-19°C at night (data not shown). The volume expansion/contraction in the displacement cylinder interferes with the set flow rate, and the net effect is significant noise as unexpected and unwanted fluctuations in the pressure drop measurements. An attempt to filter out this noise is based on calculations of the area under the curve averaged out for several days. Integration of the area under the pressure curve before day 8, gives $\Delta P_t < \Delta P_0$, which imply that the initial biological activity leads to increased permeability. After day 8, ΔP increases and the average value is positive at about 0.1 psi. Applying

equation (4), and assuming $\Delta P_0 = 0.01$ psi, the initial permeability equals 166 Darcy. An increase in ΔP to 0.1 psi implies a permeability reduction of 10 times. The ΔP monitoring correlates with the image analysis, and the numerical biomass assessments for the five sites analysed in this manner, given in Table 1. There are large variations in these measurements, with a maximum change in the image, equal to 24.2 times in position 6. The average change for the five sites from day 0 to day 8 was $N_8/N_0 = 12$. These numbers are not correlated to standard units of biomass, as it was not within the scope of this work to calibrate the method in this respect, but this is high on the list of future work. This will then be incorporated in the image analysis procedure. Optical biomass accumulation measurements should have been carried out for all the 13 selected positions. In retrospect we also see that the experiment should have continued for a longer time, increasing the support for the given statements. Observations of biomass accumulation at each specific position show that flow pattern and pore geometry are critical factors for biofilm growth, distribution and behaviour. Some observations regarding the development of biofilms in pores with different flow rates are illustrated in Figure 5. In both high and low flow rate pores biofilm accumulation went through three distinct phases: (1) ~days 1-5; smooth "homogeneous" biofilm with significant flagellar motion at the biofilm-bulk liquid interface. No detectable difference between high and low flow rate pores. (2) ~days 5-10; very little active (flagellar) motion; rougher biofilm with small and large cell clusters in the high and low flow rate zones, respectively. Channels in the biofilm in areas where the flow rate is particularly high, within a pore, developing, especially in the high flow rate pores. (3) >10 days; strands of biofilm are crossing pores producing a web-like structure. These strands initially typically evolve from "irregularities" in the pore pattern where turbulence can be observed and from ridges between the channels developed earlier. This bioweb appear to develop faster and denser in high flow zones compared to low flow zones. If this is generally the case in porous media, it has great significance for the development of flow diversion strategies, for example, to enhance oil recovery. Actual flow diversion was observed in this experiment at two sites, where in one case flow was reversed through a narrow channel towards the end of the experiment, and in the other case water started flowing through a channel which initially was stagnant.

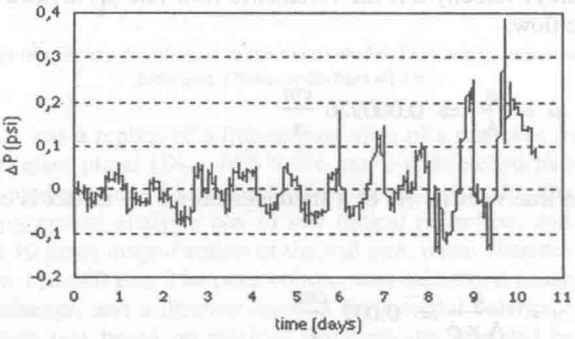

Figure 4. Differential pressure measured across the micro model.

Table 1. Numeric expression of biomass growth in selected pores. N_8 is the numeric expression of changes of the image day 8, compared to reference image, N_0

Position	Day 1*	Day 5*	Day 8*	$\frac{N_8}{N_0}$
2	0.4	3.1	6.6	16.5
4	0.43	0.92	1.5	3.5
6	0.099	2.1	2.4	24.2
9	0.35	1.4	1.6	4.6
13	0.52	0.71	5.4	10.4