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Original Article

# Biofilm Reactor Calibration for *In Vitro* Investigation of the Composite Biodegradation

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## Abstract

**Introduction:** The majority of biodegradation studies of composite materials use simplified models of microbial biofilm despite the apparent diversity of the oral microbiota. The use of in vitro systems of "artificial mouth" design is a step towards clarifying the synergistic effect that microbial plaque and human saliva have on composite degradation.

**Aim:** Establishment of functional parameters for in vitro reproduction of oral biofilms via biofilm reactor systems.

**Materials and methods:** The CDC Biofilm Reactor system consists of eight polypropylene sticks. The rod cover and the retaining plates are mounted in a 1-dm glass cylinder with an outlet side opening. The laboratory bioreactor has a working volume of 340 ml. The device is equipped with a four-blade magnetic stirrer. The system also includes gauging appliances and executive mechanisms for controlling and adjusting the basic parameters of the process.

**Results:** Determination of the operating volume of the reactor is performed prior to the experiment along with the time of reach and stabilization of the set temperature in the design which is 60 min at 120 rpm. A mathematical model is used to calculate the rate of delivery of growth medium - 11 millilitres per minute. The bioreactor is sterilized by 0.3% neomycin solution for 24 hours. Prior to the experiment the system is cleansed (via passage) with sterile water for 60 minutes.

**Conclusions:** The pre-calibration of a bioreactor system allows specification and refinement of its working parameters, thus engaging for accurate reproduction of the environmental conditions in the oral cavity.

## Keywords

artificial mouth, bioreactor, in vitro, oral biofilm

## INTRODUCTION

Oral environment is inhabited by more than 700 bacterial taxa. The hard and soft tissues in the mouth are a natural substrate used by the oral microbiota to form complex and heterogenic microcosm biofilms.[1-3]

The recent focus of contemporary cariology research is creating controllable and highly reproductive biofilm culture models via open and closed test systems. The test models based on a closed system accommodate scarce bacterial diversity and a limited supply of nutrients despite being simple to perform and cost-effective. On the other hand, open system models are more complex involving diverse bacterial spectrum and simultaneously ensuring continuous supply of fresh medium, metabolites removal, and culture liquid. Shortcomings of this type of model design are its technique sensitivity, cost, and proficiency to perform. However, open system biofilm models provide better regulation of investigation parameters and thus are rendered superior to closed system designs.[4]

The majority of studies in the literature implement simplified models of microbial biofilm despite the apparent diversity of oral microbiota. The use of artificial mouth-based in vitro design is a step towards a better understanding of the bacterial plaque-saliva complex and its combined effects on the biodegradation of composite materials.

## AIM

The aim of this study was to establish the functional parameters for in vitro reproduction of oral biofilms via biofilm reactor systems.

## MATERIALS AND METHODS

The CDC Biofilm Reactor system used in our experiment can accurately simulate an in vivo environment using computer-controlled facilities **(Fig. 1)**. It consists of eight polypropylene sticks - biofilm plate holders hinged on a polyethylene lid. The rod cover and the retaining plates are mounted in a 1-dm glass cylinder with an outlet side opening **(Fig. 2)**. The laboratory bioreactor has a working volume of 340 ml. The device is equipped with a four-blade magnetic stirrer. The system also includes gauging appliances and executive mechanisms for controlling and adjusting the basic parameters of the process. Acidity is monitored through a pH-electrode and a micro-reference electrode. Calibration processes are performed with distilled water. Environmental variables are easily controlled in the bioreactor. This allows analysis of the biofilm during its development without contaminating other samples.

Determination of the operating volume of the reactor is performed prior to the experiment along with the time of reach and stabilization of the set temperature (60 min at 120 rpm). A mathematical model is used to calculate the rate of delivery of growth medium (11 millilitres per minute). Prior to the experiment, the bioreactor is sterilized by 0.3% neomycin solution for 24 hours and cleansed (via passage) with sterile water for 60 minutes.

## RESULTS

**Table 1** presents the results of this study.

**Table 1.** Results of the present study

| Parameter | Value |
|---|---|
| Time of reach of set temperature (36.6°C) | 60 min |
| Stirring rate | 120 rpm |
| Flow of medium (BMM, species-dependent) | 11 ml/min |
| Cultivation cycle | 72 h (species-dependent) |
| Working volume | 340 ml |
| Set acidity of the environment (pH) | 7 pH |

**Figure 1.** The CDC Biofilm Reactor system simulates an in vivo environment using computer-controlled facilities.

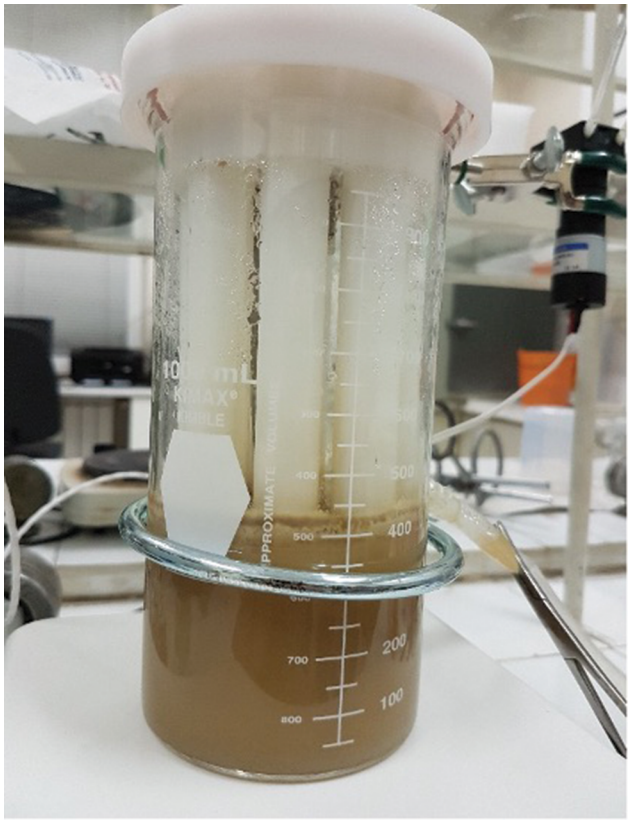

**Figure 2.** The rod cover and the retaining plates mounted in a 1-dm glass cylinder with an outlet side opening.



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## DISCUSSION

Laboratory microbial culture models simulate the oral environment for cariology studies. Unlike in vivo studies, laboratory simulations do not face problems related to the uncontrollable fluctuations of the oral environment.[5,6] Two complementary microbiological approaches can be taken to generate biofilm in microbial culture models. One of these approaches investigates the evolution of a plaque microcosm from natural oral microflora.[7] The other approach is the construction of defined-species biofilm consortia with major plaque species, or a mixture of different species of the acquired oral bacteria, such as the American Type Culture Collection (ACTT) bacteria. The designs of laboratory microbial culture models vary according to the purpose of the laboratory studies – these models are classified as closed and open systems. Each system is a compromise between the reality of the in vivo ecosystem and the simplification of the system. However, a well-designed model and a study allow researchers to obtain precise and useful results.[7]

### The closed system

Microbial culture models in the closed system have a finite supply of nutrients. The growth rates of the biofilm are rapid at the beginning of the cultivation cycle when there is plethora of nutrients. However, this is rarely observed in the in vivo growth of biofilm.[8,9] The growth conditions will change significantly following the consumption of nutrients and the accumulation of metabolic products. Hence, the physiological and biological properties of the biofilm are not comparable with those observed in vivo. Researchers use closed system models for their simplicity, high productivity, repeatability, controllability of the experimental conditions, less contamination, and cost-effective properties. The agar plate and microtiter biofilm models are two examples of a simple microbial culture model in closed system.

### The open system

The open system can be described as a continuous culture cycle system. It enables the supply of a fresh culture medium and the removal of metabolites and spent culture liquid simultaneously. Hence, the concentration of bacteria and metabolic products remains constant.[10] Moreover, the biofilm is able to stay in a stable state or in a dynamic balance.[11] Nevertheless, the repeatability of experimental result is low because of the heterogeneity of the biofilm in the open system.

The open system simulates the in vivo environment better than the closed system, allowing better regulation of the biofilm growth rate and other variables. Common microbial culture models in the open system include the chemostat model, the flow cell biofilm model, the constant depth film fermenter model, the drip flow biofilm reactor, the multiple Sorbarod model, and the multiple artificial mouth model.

The multiple artificial mouth (MAM) is a computer-controlled, multiple station model. A MAM can accurately simulate an in vivo environment using computer-controlled facilities.[12]

It has several microstations which are relatively independent to one another. Different experimental conditions can be applied simultaneously in different microstations.

Environmental variables can be easily controlled in the MAM, thus allowing analysis of the biofilm during its development without contaminating other samples. Acidity can be monitored using a pH electrode and a micro-reference electrode.[6] These well-controlled conditions improve the standardization and flexibility of the MAM, therefore enhancing its ability to culture biofilms close to natural oral flora. Sissons et al. found that biofilms developed in this system exhibited metabolic and pH behaviour that resembled typical natural plaques.[12] The MAM has been adopted in different studies, such as biodiversity of plaque, fluoride and phosphate assay[13,14], plaque calcium level measurement[14], and the generation of consortia using major plaque species[15]. The biofilm samples in this model were exposed to the same temperature and gas-phase fluctuation. The MAM aims to mimic the oral environment therefore saliva substitutes play an important role in the model. Approximate laminar flows are applied to simulate the situations in the oral cavity, instead of turbulent flow in chemostat.

## CONCLUSIONS

The pre-calibration of a bioreactor system allows specification and refinement of its working parameters, thus contributing to accurate reproduction of the environmental conditions in the oral cavity. Nevertheless, the repeatability of the experimental result is rather limited, because of the heterogeneity of the biofilm in the open system. More data need to be collected regarding the resulting biofilms, formed in a dynamic open system.

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# Калибровка биоплёночного реактора для исследования биодеградации композита *in vitro*

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## Резюме

**Введение:** В большинстве исследований биодеградации композитных материалов используются упрощённые модели микробной биоплёнки, несмотря на очевидное разнообразие микробиоты полости рта. Использование *in vitro* систем конструкции «искусственный рот» является шагом к выяснению синергетического эффекта, который микробный налёт и слюна человека оказывают на деградацию композита.

**Цель:** Установление функциональных параметров для репродукции биоплёнок полости рта *in vitro* с помощью биоплёночных реакторных систем.

**Материалы и методы:** Система CDC Biofilm Reactor состоит из восьми полипропиленовых палочек. Крышка стержня и удерживающие пластины смонтированы в стеклянном цилиндре диаметром 1-dm с отверстием на стороне выхода. Лабораторный биореактор имеет рабочий объём 340 мл. Устройство снабжено четырёхлопастной магнитной мешалкой. В состав системы также входят измерительные приборы и исполнительные механизмы для контроля и регулирования основных параметров процесса.

**Результаты:** Перед экспериментом проведено определение рабочего объёма реактора, времени достижения и стабилизации заданной температуры в расчёте, которое составляет 60 мин при 120 rpm. С помощью математической модели рассчитана скорость доставки питательной среды – 11 миллилитров в минуту. Биореактор стерилизуют 0.3% раствором неомицина в течение 24 часов. Перед началом опыта систему очищают (пассивно) стерильной водой в течение 60 минут.

**Заключение:** Предварительная калибровка биореакторной системы позволяет спецификацию и уточнение её рабочих параметров, тем самым обеспечивая точное воспроизведение условий окружающей среды в ротовой полости.

## Ключевые слова

искусственный рот, биореактор, *in vitro*, оральная биоплёнка