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Highlights:

- Applicability study of 10 surfactants for algal cultivation in liquid foam-bed PBRs
- Key surfactant properties: foaming, toxicity, biodegradability and partitioning
- Definition of suitable criteria for algal cultivation in liquid foams
- Pluronic F68: a promising surfactant to be used in algal foam-bed photobioreactors

Surfactant selection for a liquid foam-bed photobioreactor

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Abstract

A novel liquid foam-bed photobioreactor has been shown to hold potential as an innovative technology for microalgae production. In this study a foam stabilizing agent has been selected which fits the requirements of use in a liquid foam-bed photobioreactor. Four criteria were used for an optimal surfactant:~~ i)~~ the surfactant should have good foaming properties, ~~ii) it~~ should not be rapidly biodegradable, ~~iii) it~~ should drag up microalgae in the foam formed, ~~iv)~~and it should not be toxic for microalgae. Ten different surfactants (non-ionic, cationic and anionic) and two microalgae genera (*Chlorella* and *Scenedesmus*) were compared on the above-mentioned criteria. The comparison showed the following facts.~~: i)~~ Firstly, p~~P~~oloxameric surfactants (Pluronic F68 and Pluronic P84) have acceptable foaming properties described by intermediate foam stability and liquid holdup and small bubble size. ~~ii)~~Secondly, t~~T~~he natural surfactants (BSA and Saponin) and Tween 20 were easily biodegraded by bacteria within 3 days. ~~iii)~~Thirdly, f~~F~~or all surfactants tested the microalgae concentration is reduced in the foam phase compared to the liquid phase with exception of the cationic surfactant CTAB. ~~iv)~~Lastly, o~~O~~nly BSA, Saponin, Tween 20 and the two Pluronics were not toxic at concentrations of 10 CMC or higher. The findings of this study indicate that the Pluronics (F68 and P84) are the best surfactants regarding the above-mentioned criteria. Since Pluronic F68 performed slightly better, ~~we recommend~~ this surfactant is recommended for application in a liquid foam-bed photobioreactor.

Keywords: Surfactant; Microalgae; Foam-bed reactor; Liquid-foam

Introduction

In order to benefit from the great potential of microalgae for the production of specialties for food and aquaculture, biofuels and bulk chemicals, further progress is needed in the field of microalgae cultivation.[1-5] With the intention to develop a low-cost microalgae production system, a liquid foam-bed photobioreactor was developed and tested.[6] In order to create foam in the foam-bed photobioreactor, the addition of surfactants is required. Due to the continuous gas supply through a surfactant-containing media with microalgae, the generated foam is rising in the foam-bed photobioreactor, dragging the microalgal cells upwards. The foam-bed photobioreactor is illuminated and the gas phase is enriched with carbon dioxide (CO_2). As such, the microalgal cells can rapidly grow in the liquid layers within the foam. To prevent CO_2 limitation for the growing microalgal cultures, the foam has to be broken after a certain time period and regenerated with fresh CO_2-rich gas.

Previous research on the liquid foam-bed photobioreactor showed that it was possible to grow microalgae at a specific growth rate of 0.1 h^{-1}.[6] These growth experiments only lasted for 8 hours due to a decrease in foam stability in time. Since a protein, Bovine Serum Albumin, was used as a foam stabilising agent, its foamability was declining due to biodegradation and/or possible denaturation and consequent damage caused by the foaming process.[6] Long term operation of the foam-bed photobioreactor is crucial for a successful implementation of this innovative reactor system and to eventually compete with other photobioreactors. Therefore, more stable foam stabilising agents have to be selected which also match all other requirements for application in liquid foam-bed photobioreactors.

Foam stabilising agents are a highly diverse group, which is comprised of low and high molecular weight surfactants. Four different types are distinguished according to their electrostatic nature: anionic, cationic, non-ionic, and zwitterionic surfactants. In addition to this classification, synthetic and natural surfactants (e.g. proteins, saponins) are distinguished. Surfactant molecules differ in their structure, even if they belong to the same group, resulting in highly varying properties, such as foaming properties, biodegradability, and also altered interactions with algal cells.

In this study 4 specific characteristics of surfactants have been identified based on which the optimal surfactant can be selected. First characteristic is related to their foaming properties, including foam stability, liquid content, bubble size and surfactant stability. An ideal surfactant for the liquid foam-bed photobioreactor should create foams with intermediate liquid content and stability in order to create a stable, but breakable foam. Homogenous foams with small bubble sizes are preferred in order to maximize mass transfer. These properties are dependent on the surfactant structure, e.g. the length of the hydrophobic chain was shown to influence the surface activity and motility of the surfactant, determining the foam properties.[7] Moreover, in order to ensure long-term foam forming capacity, the surfactant molecules themselves must also be stable in order to be able to repeatedly form foams during long term operations of liquid foam-bed photobioreactors.

The second characteristic for surfactant selection is the biodegradation rate of the surfactant. Many surfactants can be biologically degraded,[8, 9] which means that the surfactants can be used as carbon and energy source by bacteria. However, biodegradation is directly related to the molecule's structure. For example, the more branched the carbon chain of a surfactant molecule is, the slower its biodegradation rate becomes.[10] Also the aromatic nature of a surfactant molecule can influence biodegradation rates.[11] Considering different bacteria populations grow naturally associated to growing microalgae,[12] the optimal surfactant should show a low biodegradability in order to minimize surfactant losses associated to its bacterial consumption.

The third characteristic is the partitioning of microalgae to the foam phase. It is evident that the foams formed by the chosen surfactant must be enriched with microalgae. Microalgal partitioning towards the foam is determined primarily by hydrostatic interaction between the air bubbles and the microalgae, thus algal cell surface hydrophobicity might play a role.[13] Besides, the electrostatic charging of the algal cells and the surfactant molecules are crucial. Surfactants can also render algal cell surface hydrophobicity by attaching to cells via electrostatic interactions,[14] or alternatively the bubbles can own an electrostatic charge due to the charged surfactant molecules used for foam stabilisation.[15] Cationic surfactants, for example, have a higher algal foam partitioning as they attach the strongest to the usually negatively charged algal cell.[14]

The fourth characteristic of a surfactant which is essential for its application in a liquid foam-bed photobioreactor is its toxicity to microalgae. The reported toxic effects of a surfactant on microalgal growth are a reduction in cell density, growth rate, cell motility, chlorophyll content, and the inhibition of the photosynthetic activity.[16-19] The extent of this toxicity is dependent on surfactant type, concentration, and the microalgal species involved.[20] For example, cationic surfactants are, in general, more toxic than their anionic or non-ionic partners.[21]

Although some basic principles are understood, it is still difficult to predict surfactants properties and their interactions with different algal species solely based on their structure and theory. The aim of this study is therefore to empirically select a suitable foam stabilising agent for the liquid foam-bed photobioreactor that can enable elongated cultivations of days to weeks. In the current study 10 different surfactants were evaluated in terms of measured foaming properties, biodegradability, algal partitioning towards the foam and toxicity to microalgae. For this, *Chlorella sorokiniana* and *Scenedesmus obliquus* were used according to their robustness and diverse array of potential applications.[22, 23] Based on these results the best surfactant for the application in foam-bed photobioreactors was selected.

Materials and methods

Surfactants

Ten different surfactants were evaluated for their suitability for the liquid foam-bed photobioreactor. The selected surfactants contain two natural surfactants and 8 synthetic surfactants: six non-ionic (Pluronics F68 and P84, Tergitols NP9 and TMN6, Triton X-100 and Tween 20), one cationic (CTAB), one anionic (SDS). The natural surfactants were Saponin and a reference protein surfactant, Bovine Serum Albumin.[6] All the surfactants used are listed in Table S1. The comparison of these surfactants was done at surfactant specific concentrations, taking into account their critical micelle concentration (CMC). The CMC is the concentration above which the surfactant molecules start forming micelles in the liquid phase. The CMC values of the different surfactants are included in Table S1. These CMC values were collected from literature and/or the product information sheets.

Microalgae, cultivation media and cultivation conditions

Chlorella sorokiniana (CCAP 211/8K) was obtained from the Culture Collection of Algae and Protozoa, Oban, Scotland *and Scenedesmus obliquus* from the Culture Collection of Fitoplancton Marino S.L., Cádiz, Spain.

For the algae partitioning assays, *C. sorokiniana* was grown on 3 times concentrated M8a media, in which the N and P content were further modified to enable high biomass densities compared to the M8a recipe described previously[24] (see Supplementary material). The growth medium for *S. obliquus* was based on the medium from Breuer et al., [25] but further modified in order to reach higher biomass densities. Both microalgae were grown in 250-300 mL shake flasks placed in an orbital shaker in an incubator. The incubator settings were the following for *C. sorokiniana*: 37 °C, 454 µmol $m^{-2} s^{-1}$, 120 rpm and 4 % CO_2, and for *S. obliquus*: 25 °C, 120 µmol $m^{-2} s^{-1}$, 80-100 rpm and 2.5 % CO_2.

The culture media employed for the toxicity assays of surfactants to microalgae were M8a media[22] with 3 g L^{-1} of urea for *C. sorokiniana,* and modified BG11 media for *S. obliquus.*[26] For the biodegradability assays, *S. obliquus* was maintained in a modified M8a medium in which urea was replaced by KNO_3 (3 g L^{-1}) in order to avoid the presence of any other carbon source in the media apart from the surfactants. Unless otherwise indicated, for the biodegradability and toxicity experiments both strains were maintained in linear phase cultures in shake flasks placed in a growth chamber at 25 ºC. The cultures were continuously illuminated at 80 µmol photons m^{-2} s^{-1} with fluorescent lamps and bubbled with air enriched with 2.5-% (v/v) CO_2.

Surfactant foaming properties

Foaming properties of the different surfactant solutions were determined by an automatized foaming device (FoamScan, Teclis- IT Concept, Logessaigne, France), as described previously.[6] Foam stability is measured in terms of the time elapsed until half of the foam volume had collapsed, and this will be referred to as the foam half-life. The liquid holdup of the foam represents the amount of liquid incorporated in the foam at the moment when the foam reached its desired volume and the gas supply stopped. All experiments were performed at a temperature between 28 and 30 °C. The gas flow rate employed was 400 cm^3 min^{-1}, resulting in 2.4 mm s^{-1} superficial gas velocity. Two different concentrations were used for each surfactant: 1 and 5 CMC. BSA was measured at 1 g L^{-1} and 5 g L^{-1} at higher temperatures of 37 °C.[6] The experiments were performed in duplicates.

The bubble size was analysed from pictures taken 30 seconds after the gas flow had stopped, at a height of 8 cm above the gas distributor. The mean bubble size and the standard deviation were calculated by an image analysis software (Foamscan) for each measurement, and the standard deviations and the average of the means of the two independent measurements were calculated.

The stability of the surfactants during repetitive foaming was assessed by comparing the up-flow speed of foams formed by fresh surfactant solution and the same solution after 20 continual cycles of foam production and subsequent deconstruction. Foam was produced from 200 mL surfactant solution in a 1 L glass graduated cylinder by distributing air through two small-bubble diffusers at a superficial velocity of 3.68 mm s^{-1}. Surfactant solutions were prepared in M8a culture medium adjusted to pH 6.8. Foam up-flow speeds were calculated for a 30 seconds foaming time by measuring the foam level. Each surfactant test was performed in a maximum of 10 hours to prevent losses of foaming properties due to other causes (e.g., biodegradability, light sensitivity). The minimum surfactant concentration resulting in maximal foam-up flow speed (C_{min}) was determined and 1.1 times C_{min} was used in the stability tests. C_{min} values (expressed in terms of CMC) found for the different surfactants were: BSA, 5; CTAB, 0.5; Pluronic F68, 5; Pluronic P84, 4.25; Saponin, 0.5; SDS, 0.08; Tergitol NP9, 1; Tergitol TMN6, 1; Triton X-100, 0.75, and Tween 20, 0.5.

Surfactant biodegradability

The biodegradability of the surfactants was indirectly determined by following bacterial growth. Bacterial growth was studied at 1 and 10 CMC surfactant concentrations, since in a foam-bed photobioreactor, concentrations within these limits are expected. The concentration of 1 CMC still allows for acceptable foam formation, and possible inhibitory effects on bacterial cells are minimized. A high concentration of 10 CMC was chosen in order to assess promoting and/or inhibiting surfactant effects on bacterial growth. The experiments were performed in duplicates in 100 mL shake flasks. Surfactant solutions were prepared in M8a media, with nitrate as nitrogen source, and they were sterilized by filtration. Each surfactant solution was inoculated with 10 mL of *Scenedesmus* culture supernatant, which naturally contains microalgae-associated bacteria. The cultures were placed in an orbital shaker at 37 ºC and 115 rpm. Bacterial growth was measured as the increment of optical density at 600 nm

($OD_{600}^{culture}$) after 3 days (t_3), which was normalized by the initial optical density of each solution ($OD_{600,t0}^{culture}$), as shown in Equation 1. In order to avoid errors related to turbidity changes in the surfactant solutions during the experimental time, blank cultures with the different surfactant solutions (not inoculated) were prepared (OD_{600}^{blank}).

$$\boldsymbol{OD\ increase\ factor} = \frac{(OD_{600,t3}^{culture} - OD_{600,t3}^{blank}) - (OD_{600,t0}^{culture} - OD_{600,t0}^{blank})}{(OD_{600,t0}^{culture} - OD_{600,t0}^{blank})} \qquad \text{Equation 1}$$

Microalgae partitioning towards the foam phase

In this study the partitioning of microalgae towards the foam (P_x^{foam}) was expressed as the ratio of the microalgae concentration in the foam liquid phase ($C_{x,}^{foam}$) over the initial microalgae concentration in the liquid phase used to form the foam ($C_{x,}^{initial}$), as presented in Equation 2.

$$P_x^{foam} = \frac{C_x^{foam}}{C_x^{initial}} \qquad \text{Equation 2}$$

For the definition of P_x^{foam} the initial biomass concentration is considered instead of the concentration in the bulk liquid since the bulk liquid biomass concentration is continuously changing with increasing foam volume. This definition of P_x^{foam} allows for comparison between different systems with different dimensions.

Microalgae-surfactant mixtures were prepared by adding the surfactant to the microalgal culture (*C. sorokiniana* or *S. obliquus*). This mixture was foamed in a 2.5 L glass tank (20.5~~x~~×3~~x~~×40 cm, width ~~x~~× depth ~~x~~× height). Silicon tubing (internal diameter of 4 mm, wall thickness of 2 mm) punctured by a 0.45 mm needle at 1.4 cm intervals served as gas distributor. This tubing was placed one cm above the bottom of the glass tank over the complete width. A gas flow rate of 1000 mL~~.~~ min^{-1} was supplied to the surfactant solutions containing microalgae to allow foam formation. The volume of the algae suspension was 200 mL for *C. sorokiniana* experiments, and 100 mL for *S. obliquus*. The difference in the volumes was due to the fast settling of *S. obliquus* cells underneath the gas distributor. For this reason, the volume underneath the gas distributor was filled with 100 mL of solidified Agarose gel (5–%), and only 100 mL algae suspension was used to keep the same liquid height before foaming. The surfactant concentration was 5 CMC in order to ensure sufficient foam formation, except for Saponin where 5 CMC did not generate enough foam for the measurements; thus, 10 CMC was used. BSA was tested at a concentration of 1.75 g L^{-1}, which represents the concentration applied previously in a liquid foam-bed photobioreactor.[6] For each surfactant, duplicate foaming experiments were performed.

The experiments with *C. sorokiniana* were started at an optical density (OD) of 15 to 20 (i.e. $C_{x,}^{initial}$), while the experiments with *S. obliquus* were started with an OD of 2. Foam was allowed to rise in the glass tank, and the foam flowing out on top was collected. In each experiment, four different foam samples of 150 mL were taken, provided sufficient foam was formed. The first 150 mL of foam was discarded and the remaining three foam fractions were allowed to collapse in cups. A sample was taken from the collapsed foam and the OD was measured in duplicate. Afterwards, these OD values were averaged for the three different foam fractions, and the two duplicate experiments. The optical density was measured at 750 nm in a spectrophotometer (Hach Lange DR6000, Germany). As SDS solutions were opaque the cell concentration was determined by cell counting: microscopic cell count using counting chamber and automatic cell counting with Beckman Coulter Multisizer 3 employing a 50 µm aperture tube. In order to evaluate the differences in partitioning between the different surfactants, t-tests were done with individual pairs.

Toxicity of surfactants to microalgae

The toxic effects of surfactants on the selected microalgal strains were tested at the following surfactant concentrations: 0.1, 0.5, 1, 2.5, 5, 10, 15, 25 and 50 CMC, provided the surfactant was still soluble at that concentration. Cultures were prepared in shake flasks containing 300 mL of surfactant solution prepared in the corresponding microalgal culture medium and inoculated with microalgae cells growing in linear phase. Microalgal cultures were prepared in triplicate under sterile conditions and biomass concentration was adapted in order to get an initial optical density of 0.8 at 750 nm. The cultures were not aerated in order to prevent foam formation. Instead of bubbling, the cultures were magnetically stirred at a speed of 200 rpm in order to maintain the homogeneity of the suspensions. Optical density at 750 nm (UV/ Visible spectrophotometer, Evolution 201, ThermoFisher, United States) and maximal photosynthetic efficiency of Photosystem II (AquaPen-C AP-C 100, Photon Systems Instruments, Germany) were measured daily for a minimum of 3 days.

Results and Discussion

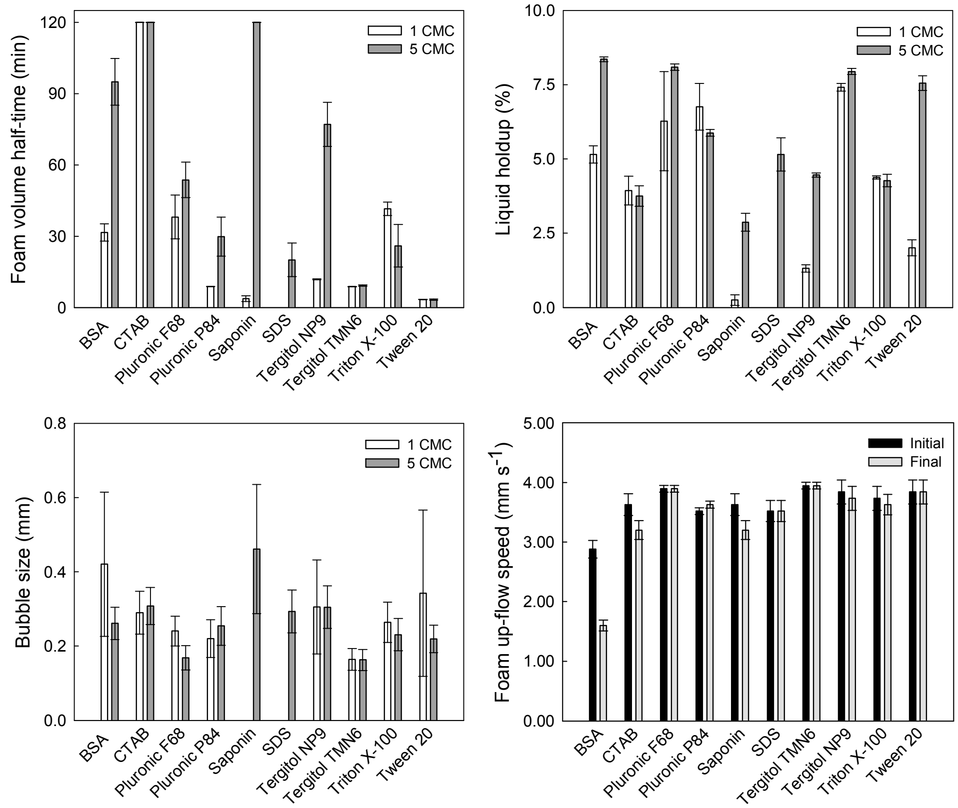
Surfactant foaming properties

In order to determine the foaming properties of the different surfactant solutions, foam volume half-life, liquid holdup in the foam, and bubble size of the foam were analysed for ten surfactants at two different concentrations, 1 CMC and 5 CMC. A concentration of 1 CMC is the minimal concentration required in order to reach the maximal reduction in surface tension and, as such, good foaming characteristics.[27] A concentration of 5 CMC was also used to ensure that depletion does not occur in the liquid phase (>1 CMC). From the data in Figure 1A, it is apparent that at a surfactant concentration of 5 CMC, CTAB and Saponin formed the most stable foams. The foam volume half-life was more than 2 hours for these surfactants. The exact half-life value was not determined since the experiments were terminated after 2 hours. Besides CTAB and Saponin, long foam volume half-life was observed for BSA and Tergitol NP9 at 5 CMC concentration. In contrast, Tergitol TMN6 and Tween 20 foams showed rapid destabilisation, as exemplified by a foam half-life of less than 10 minutes at 5 CMC. The surfactant SDS also resulted in unstable foams and at 1 CMC it did not reach the foam height required for the analysis.

Most surfactants showed increased foam stability at 5 CMC in comparison to 1 CMC (Figure 1A). Since the surface tension of a surfactant solution does not change above CMC, the foaming properties are also expected to be unchanged.[28] The decrease in stability when decreasing the concentration from 5 to 1 CMC might be due to the depletion of surfactant molecules from the liquid phase at 1 CMC. The surfactant molecules, due to their amphiphilic nature, partition to the foam phase when gas is introduced to the solution, thus the amount of surfactant molecules is reduced in the bulk liquid remaining underneath the foam. The depletion effect is especially relevant when large foam volumes are formed, or the initial surfactant concentration is low.[29] Furthermore, the rate of diffusion of the surfactant molecules to the interface also influences the foam stability and thereby might result in further foam stability increase above CMC. When the surfactant transport rate is slow, foam will be formed with reduced amount of adsorbed surfactant molecules, thus the foam stability will also be reduced.[30] Finally, the change in foaming properties above CMC might be explained by further foam stabilisation due to the presence of micelles in the thin films.[31] Consequently, at higher surfactant concentrations (>CMC) more stable and more wet foam can be formed.

The liquid holdup measured in the foam is presented in Figure 1B. The highest holdups were obtained with BSA, Pluronic F68, Tergitol TMN6 and Tween 20 at 5 CMC. These foams had a liquid holdup between 7.5 and 8.5%. Under the same conditions (5 CMC), the lowest liquid holdup was 2.9% for Saponin foams. As illustrated in Figure 1B, the liquid content of the foam is influenced by surfactant concentration,[32] with generally lower liquid holdup at 1 CMC in comparison to 5 CMC. As explained before this effect is probably related to surfactant depletion, surfactant diffusion limitation or micelle-based stabilisation. Next to foam stability and liquid holdup, the bubbles sizes were analysed (Figure 1C). In general, an increased surfactant concentration leads to a small reduction in bubble size, although for some surfactants the bubble size did not change with surfactant concentrations above CMC. Tergitol TMN6 formed foams with the smallest bubble sizes, below 0.2 mm radius. The largest bubbles were formed by Saponin at 5 CMC, with almost 0.5 mm average bubble radius. The standard deviations in Figure 1C can be regarded as a measure of the uniformity of the foam: bigger error bars mean a more heterogeneous foam, while at homogeneous foams the error bars are minimal. Generally, foams with high bubble size uniformity are more stable due to the reduced Laplace pressure between the adjacent bubbles.[33] When comparing the different surfactants, generally smaller bubble sizes were related to increased liquid holdup values and this correlation was even more dominant when considering only one surfactant at the two different concentrations (Figure 1B and 1C). Therefore, we can conclude that increasing surfactant concentration results in the combined effects of decreased bubble size and increased liquid holdup and foam stability, corresponding to previous observations.[34]

Besides foam stability, liquid content and bubble size, surfactant stability against foaming is an important aspect. In previous research it was shown that BSA is not able to produce foams for elongated time periods.[6] Now we show that this phenomenon is not necessarily due to bacterial biodegradation of the molecule, but is more likely related to the instability of the surfactant during continuous foaming. Figure 1D shows the initial and final foam up-flow speed of each surfactant after 20 cycles of foam production and deconstruction. A reduction in this parameter could denote a loss of surfactant foamability, and therefore, of surfactant stability. The evolution of the foam up-flow speed during the 20 cycles (data not shown) revealed a dramatic drop of the up-flow speed of BSA within a short time range, with a reduction of 56% of the original value after the first three cycles. The mechanism behind the reduction of BSA foam stability is suggested to be a consequence of conformational changes in the molecule during foaming.[35] For all other surfactants no significant decrease in foam stability was observed after 20 foaming cycles.



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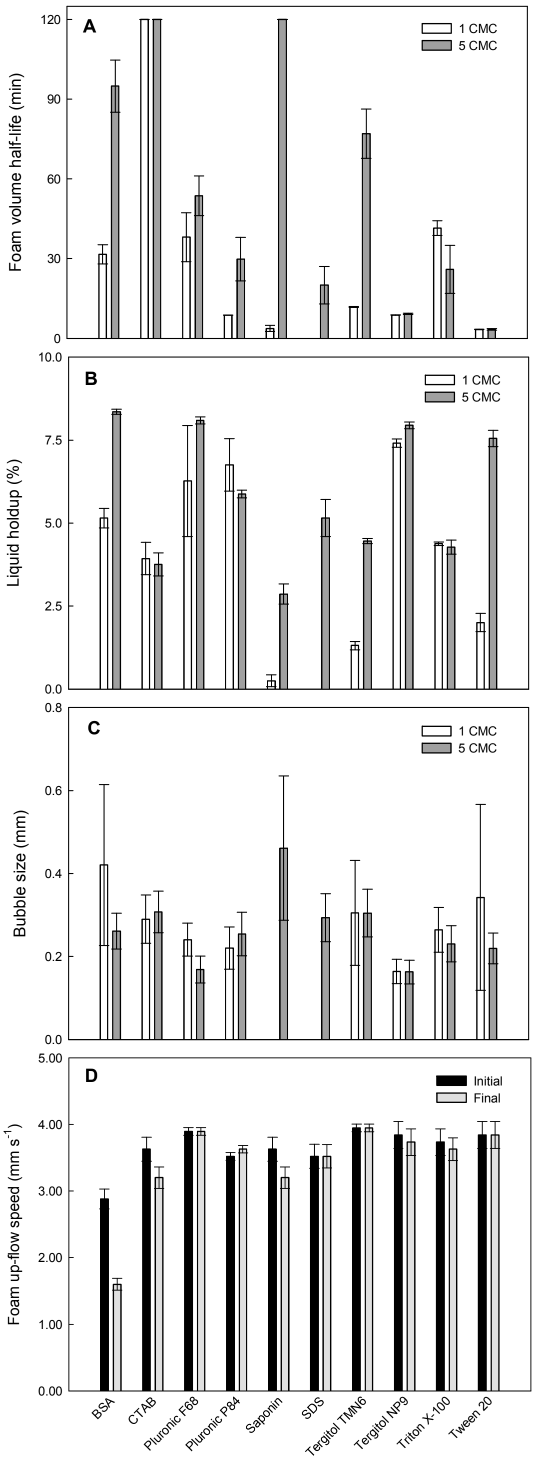


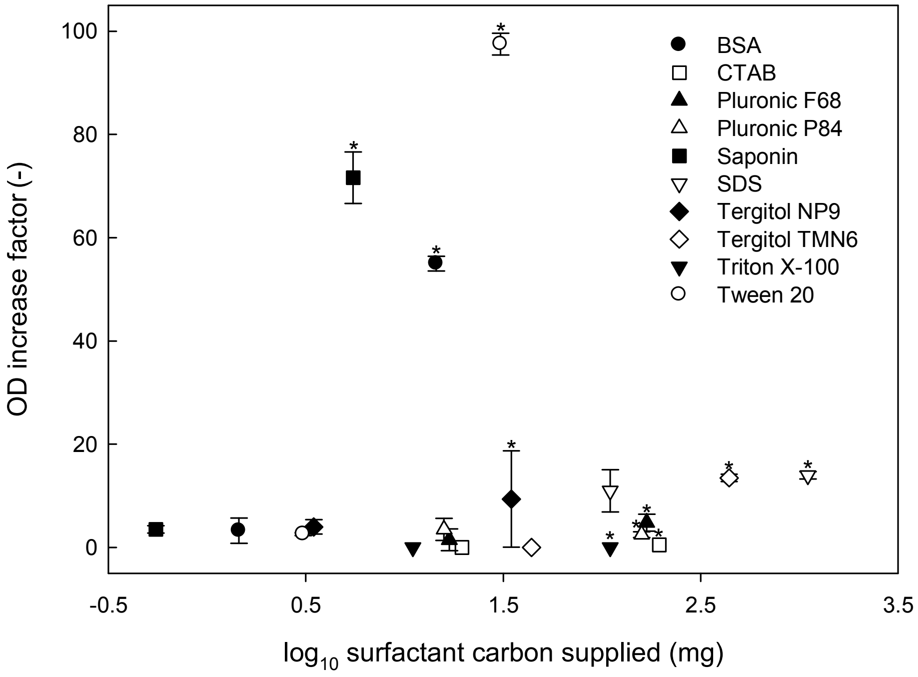
Figure 1. Surfactants foaming properties: A) Foam stability, represented in terms of foam

volume half-life, which is the time elapsed when the foam volume has collapsed to its half. B) Liquid holdup in foams stabilized by different surfactants, expressed as the volume percentage of liquid incorporated in the foam. C) Bubble size of foams formed by different surfactants. D) Surfactants stability, represented as the initial and final foam up-flow speed of surfactant solutions after 20 cycles of repeated foam formation and deconstruction. Parameters A, B and C were analysed at two different surfactant concentrations (1 CMC and 5 CMC). SDS and Saponin at 1 CMC did not form sufficiently stable foams for the analysis of bubble size. Liquid holdup and foam stability could not be measured with SDS at 1 CMC due to its low foaming ability. The error bars represent the standard deviation of the measurements.

Surfactant biodegradability

In order to select the most suitable surfactant, its susceptibility to bacterial degradation (i.e. biodegradation) was determined. This was done by following the growth of the inoculated bacteria (obtained from the supernatant of a *S. obliquus* culture) in the fresh water algal culture medium enriched with each surfactant at concentrations of 1 and 10 CMC. The surfactants added were the only reduced carbon source available to promote bacterial growth. Thus, bacterial growth, if observed, was a direct evidence for bacterial degradation of the surfactants as source of carbon and energy. Microalgal consumption of surfactants was not investigated since it was shown previously that BSA, one of the most easily biodegradable surfactants, was not consumed neither adsorbed by microalgal cells.[6] Therefore, the algal degradation of surfactants was considered negligible compared to bacterial degradation. Algal cells were removed from the inoculum, since decaying algal biomass due to the absence of light or surfactant toxicity might result in additional carbon source for bacterial growth.

Figure 2 shows results of bacterial growth on the different surfactants after 3 days of cultivation at 2 different concentrations (1 and 10 CMC). In order to strictly compare the surfactants in terms of the carbon content supplied, the X-axis is expressed as the decimal logarithm of the milligrams of carbon added to each culture, calculated from the carbon content of the surfactants. Cultures which showed bacterial growth reached the stationary phase and cultures that did not show any growth remained unchanged even after 2 weeks (data not shown). According to the figure, the lowest bacterial growth was obtained in the culture medium containing either CTAB, both Pluronics, or Triton X-100. For these surfactants no differences were observed for the two concentrations tested. Despite their high carbon content, compared to the other evaluated surfactants, the bacterial growth was minimal and, therefore, they can be considered less susceptible to biodegradation. On the other hand, the bacterial growth was by far more intense in the cultures containing BSA, Saponin and Tween 20 at 10 CMC, suggesting that indeed the carbon contained in these compounds was used as substrate for bacterial growth. A third group with surfactants which showed an intermediate biodegradability could be inferred from the figure. In this sense, both Tergitols and SDS promoted moderate bacterial growth. Bacterial growth was independent of the initial carbon content when SDS was used, which might indicate low efficiency of bacteria to degrade such surfactant and/or a possible toxic effect. Finally, regarding both Tergitols, a higher concentration of TMN6 was needed to promote the same bacterial growth as NP9, which might indicate Tergitol NP9 is slightly more readily biodegradable.



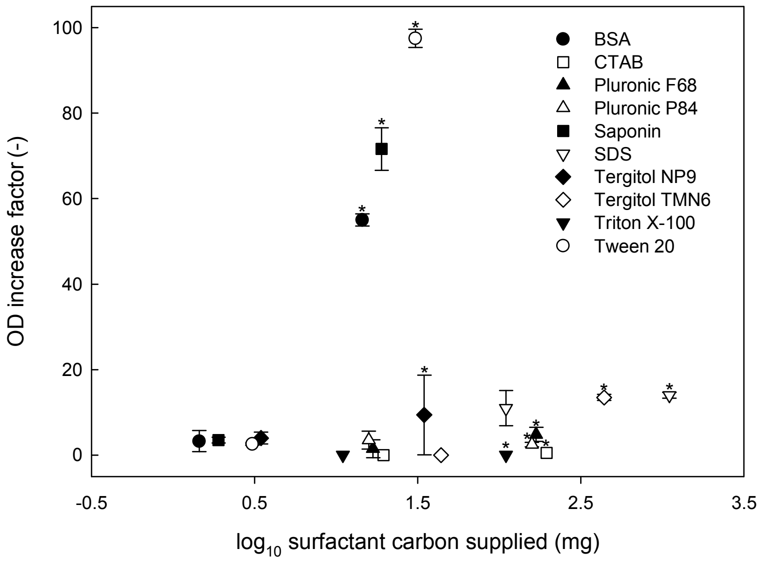


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Figure 2. Surfactants biodegradability, represented as the bacterial growth on surfactant solutions after 3 days of cultivation at 1 and 10 CMC surfactant concentrations. The "*"

indicates data obtained at 10 CMC. Bacterial growth is expressed as the increase in optical density (600 nm) after 3 days and it is plotted against the carbon dosage resulting from surfactant addition (logarithmic values, in milligrams). Optical density values were corrected with their corresponding blanks (culture medium with surfactant) and optical density increments were normalized with respect to their initial optical densities.

The bacterial ability to degrade a surfactant and use it for growth depends on the chemical structure of the surfactant.[9] Three of the evaluated surfactants have fatty-alcohol ethoxylate structure (FAE; commercial names: Pluronic F68, Pluronic P84, Tergitol TMN6), and two of them have alkylphenolpolyethoxylate structure (APE; commercial names: Tergitol NP9 and Triton X-100). Under aerobic conditions, most surfactants can be degradable, including FAE and APE, and thus can favour bacterial growth.[36] However, there are differences between surfactant families regarding their degradability. For instance, according to the obtained results Triton X-100 (APE) is less readily biodegradable than SDS (alkylbenzenesulphonate), which is in agreement with previous observations.[37] The linear structure of SDS, compared to the branched and the ethoxylated chain of Triton X-100, may be among the reasons that explain its higher biodegradability.[38]

Microalgae partitioning towards the foam phase

Microalgae partitioning to the foam phase, P_x^{foam}, has been measured and the results are presented in Figure 3. A partitioning of 1 means that the algae concentration in the foam liquid is identical to the initial concentration in the liquid prior foam formation, implying that it is also identical to that in underlying bulk liquid as can be calculated from a microalgae mass balance over the system. Detailed explanations about these mass balances are presented in the Supplementary Material. The first foam layer (150 mL) exiting the vessel showed large deviations in algae concentration compared to the following three foam fractions, thus it was not considered in the calculation of the P_x^{foam} values. This deviation might be due to foam destabilisation at the top of the foam column and cells not draining efficiently through the foam layer as liquid drains.[39, 40]

Only the cationic surfactant (CTAB) showed a partitioning higher than 1. Microalgae cells concentrated in the CTAB stabilized foams, since the foam had 6.8 (*C. sorokiniana*) and 1.4 (*S. obliquus*) times higher algae concentration compared to the original algae solution. This is reasoned by electrostatic interactions between cells and surfactants,[41, 42] since cationic surfactants show a strong electrostatic interaction with the negatively charged microalgae cells,[14, 43] as described for foam flotation systems. For all the other surfactants the partitioning factor was below 1 meaning that the algae have a preference to remain in the bulk liquid and not in the foam liquid. Despite the differences among those were not statistically significant, the natural surfactants (BSA and Saponin) and the non-ionic surfactant Triton X-100 showed slightly higher algae partitioning for the foam phase compared to the rest of the surfactants. The t-tests showed that these surfactants have significantly higher partitioning compared to most other surfactants, but not all. The 6 remaining surfactants (5 non-ionic, 1 anionic) showed similar P_x^{foam} values. The anionic surfactant SDS did not result in the lowest partitioning for *C. sorokiniana*, however *S. obliquus* showed the lowest P_x^{foam} results with SDS, possibly due to the electrostatic repulsion forces between the surfactant and the algal cell surface. As non-ionic surfactants do not own electrostatic charge, possibly hydrophobic interaction took place between the algal cell membranes and the surfactant.[44] Thus, the difference in algal partitioning between the different non-ionic surfactants might be due to their difference in hydrophobicity, affecting their efficiency of adsorption.[42]

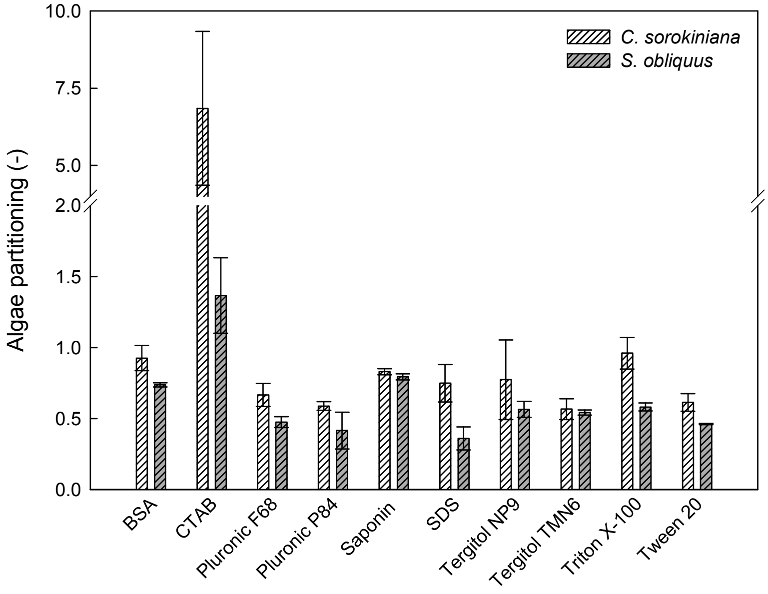

Figure 3. *Chlorella sorokiniana* (white hatched bars) and *Scenedesmus obliquus* (grey hatched bars) partitioning towards the foam phase. The partitioning is defined as the ratio between the algae concentration in the foam and the algae concentration of the suspension before foaming. The experiments were carried out at the surfactant concentration of 5 CMC (except for Saponin where 5 CMC did not generate enough foam for the measurements; thus, 10 CMC was used). BSA was tested at a concentration of 1.75 g L^{-1}. The microalgae concentration was 15-20 expressed in OD at 750 nm for *C. sorokiniana* and 2 for *S. obliquus*.

In general, *C. sorokiniana* had a higher partitioning to the foam compared to *S. obliquus*. The differences in P_x^{foam} between the two strains can be due to differences in the initial cell concentration applied, but also due to differences in cell surface hydrophobicity,[45] since hydrophobic-hydrophilic interactions are determining the attachment of particles to air bubbles[46] and to surfactants.[44] Besides cell surface hydrophobicity, cell size and shape of the microalgae might have an influence on their partitioning: *S. obliquus* cells are long, narrow cells and larger in size than the small spherical *C. sorokiniana* cells.

Toxicity of surfactants to microalgae

The toxicity of the surfactants to *Chlorella sorokiniana* and *Scenedesmus obliquus* was studied by analyzing the effect of the surfactants on microalgal growth and photosynthetic efficiency (Figure 4). A surfactant concentration was considered toxic when a 10~~-~~% reduction (dashed line in Figure 4), or higher, was found in optical density and/or photosynthetic efficiency with respect to the control cultures without surfactants. Although toxicity experiments were done at several different surfactant concentrations, Figure 4 represents only the points around the highest concentration without inhibitory effect and the lowest toxic concentration.

The toxicity results, considering the surfactant concentration used in weight per volume units, showed different sensitivity of each microalga to a given surfactant. In general, *Scenedesmus obliquus* cultures (Figure 4 C-D) seem to be slightly less sensitive to evaluated surfactant concentrations than *Chlorella sorokiniana* (Figure 4 A-B) as similar toxicity is produced at higher concentrations of most surfactants. The reason behind the species-dependent toxic effects of a certain surfactant might lay in the differences in algal cell wall composition between species.[47] Moreover, surfactants with different chemical structure, even owning the same electrostatic character (e.g. non-ionic), may exhibit different toxicity to a given microalga species. For instance, this is the case for both Pluronics, which exert different toxic effects on *S. obliquus* (Figure 4), Pluronic F68 being less toxic than Pluronic P84. Toxicity of poloxamers has been reported to be proportional to their lipophilic character,[48] which is in agreement with our findings. One of the main reported effects of the surfactants is the cell surface depolarization[49] which has the consequence of decreasing nutrient consumption, therefore affecting algal growth negatively. The alterations of cell surface properties are related to the integration of alkyl chains into the cell wall.[50]

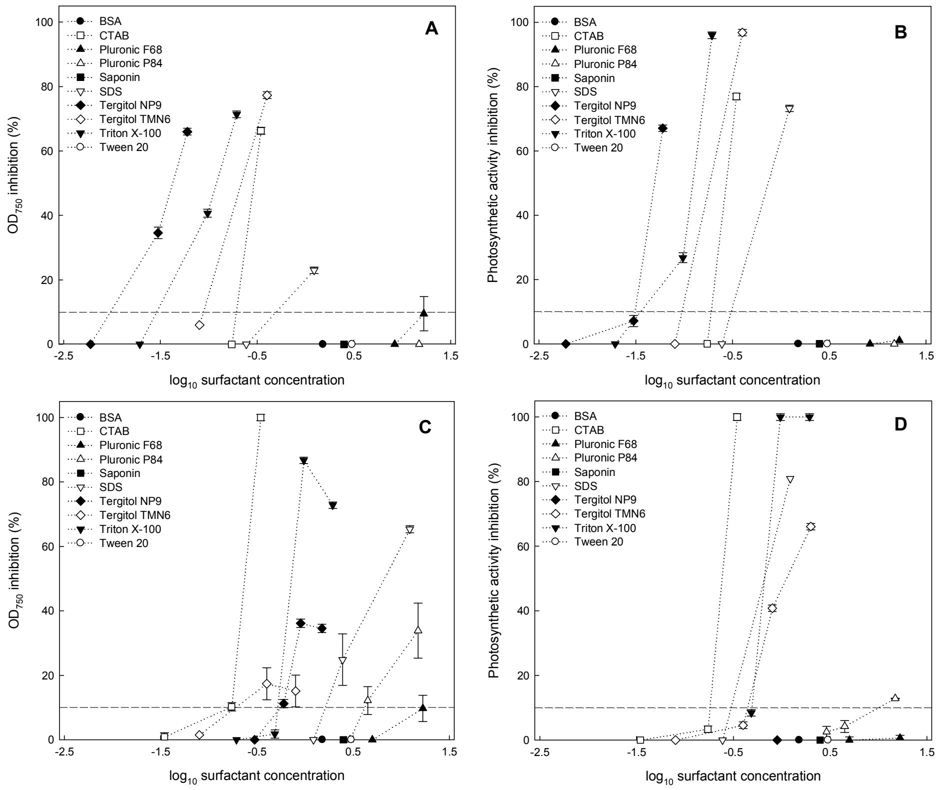

Figure 4. Surfactants concentration-response curves for *C. sorokiniana* (A-B) and *S. obliquus* (C-D) after 72 hours of exposure in liquid cultures. The ordinate indicates the percentage of inhibition in the biomass growth (represented as optical density at 750 nm) (A, C), and in the maximum photosynthetic efficiency of Photosystem II (B, D) in dependence of the logarithm of the surfactant concentration used in grams per litre. The dashed line indicates the 10–% inhibition threshold.

According to their toxic effects on the selected microalgae, the evaluated surfactants could be divided in two main groups. The two natural surfactants (BSA and Saponin), the two Pluronics (F68 and P84), and Tween 20 showed the lowest levels of toxicity to the tested microalgae species. On the opposite side, the ionic surfactants (CTAB and SDS), both Tergitols (NP9 and TMN6) and Triton X-100 exhibited the highest levels of toxicity to both microalgae. Altogether, it can be inferred that the toxicity of surfactants to microalgae depends on the specific chemical structure and concentration of the surfactant but it is also species-dependent.[36, 49]

Surfactant selection

In order to select the most appropriate surfactant for the liquid foam-bed photobioreactor, the foaming properties, biodegradability, microalgae partitioning to the foams, and toxicity have to be evaluated together.

The optimal surfactant for the liquid foam-bed photobioreactor should be capable of forming large foam volumes, with small and uniform bubbles. Small bubbles are relevant for increased mass transfer in the foam-bed photobioreactor due to the increased surface area. Further advantage of small bubbles is their increased particle flotation efficiency, possibly resulting in an increased algae partitioning towards the foam phase.[51] On the one hand, the formed foam should not be too stable (>2 h, e.g. CTAB and Saponin at 5 CMC) in order to facilitate foam break-up. Fast destabilisation, on the other hand, as observed with Tween 20 and Tergitol TMN6, might result in foam collapse already while the foam is rising in the reactor, resulting in heterogeneous, coalescing foams. This would be especially problematic at low gas flow rates or high foam-bed photobioreactors. Besides, the liquid content of the foam should be intermediate in order to achieve increased biomass concentration compared to conventional photobioreactors, while still maintaining high ground-areal and volumetric productivities.

Altogether, the following ranges were identified for the different parameters defining the foaming properties: the liquid holdup of surfactants should be between 5-10%, the foam volume half-life should be between 25 and 120 minutes, the bubble radius should be smaller than 0.4 mm, and surfactants should show a constant foam up-flow speed during at least 20 cycles. As shown in Figure 1, surfactants with a higher liquid holdup than 5% were BSA, Pluronic F68, Tergitol TMN6, Tween 20, Pluronic P84 and SDS at 5 CMC. Surfactants with intermediate foam stabilities were BSA, Tergitol NP9, Pluronic F68, Pluronic P84 and Triton X-100. The bubble size was too large for Saponin, all other surfactants fell in the good range. The protein BSA did not show sufficient stability against foaming.

Regarding surfactant biodegradability, the selected surfactant must not be easily degraded by the bacterial populations that are naturally associated to the algal culture, since the use of a readily biodegradable foam stabilising agent would imply a relative rapid increase of bacterial populations in the algal cultures, together with the need of a continuous addition of surfactant to maintain its concentration constant in the liquid foam-bed photobioreactor. In this study, 3 of the 10 surfactants tested (BSA, Saponin and Tween 20) were easily biodegraded at surfactant concentrations higher than 1 CMC. Considering the foam requirements, the surfactant concentration needs to be above 1 CMC and, therefore, these surfactants are discarded for their use.

For the liquid foam-bed photobioreactor, maximal partitioning of microalgae to the foam phase is preferred. Elevated algae concentrations in the foam phase result in an increased fraction of algal cells exposed to illumination, therefore contributing to higher growth rates and reactor productivity. Due to the generally low algae partitioning of the non-ionic and anionic surfactants and the relatively small difference between their partitioning values, the cationic surfactant CTAB, Triton X-100, BSA and Saponin were considered to show best performances.

Last, but not least, a pre-requisite of a surfactant to be employed for algal cultivation in foam is to be non-toxic for the alga cultivated.In general, most of the surfactants showed toxicity below 10 CMC (Table 1). The cationic surfactant CTAB, the anionic surfactant (SDS), and both Tergitols resulted as the most toxic surfactants for both strains when comparing in terms of CMC. In this sense, the criterion for a non-toxic surfactant is the absence of toxic effects (no reduction of growth rate nor photosynthetic efficiency) at the concentration of 10 CMC. According to this, only both Pluronics and BSA, Saponin and Tween 20 fit the requirement. For the cultivation of other strains, the tolerance of the two species investigated to the given surfactants cannot be extrapolated to others and should be specifically studied.

Table 1. Experimental m~~M~~aximum non-toxic surfactant concentration tested ~~with no toxic effects~~ for the microalgal strains *C. sorokiniana* and *S. obliquus*, expressed in function of their CMC values. CMC values for the surfactants used in the experiments are included in Table S1 as supplementary material.

~~-~~	~~-~~	~~Experimental maximum non-toxic surfactant concentration (CMC)~~								
	~~BSA~~	~~CTAB~~	~~Pluronic F68~~	~~Pluronic P84~~	~~Saponin~~	~~SDS~~	~~Tergitol NP9~~	~~Tergitol TMN6~~	~~Triton X-100~~	~~Tween 20~~
~~*C. sorokiniana*~~	~~50~~	~~0.5~~	~~50~~	~~50~~	~~50~~	~~0.1~~	~~0.1~~	~~0.1~~	~~0.1~~	~~50~~
~~*S. obliquus*~~	~~50~~	~~0.1~~	~~50~~	~~10~~	~~50~~	~~0.1~~	~~5~~	~~0.1~~	~~2.5~~	~~50~~

Algal strain	-	**Experimental maximum non-toxic surfactant concentration (CMC)**								
	BSA	CTAB	Pluronic F68	Pluronic P84	Saponin	SDS	Tergitol NP9	Tergitol TMN6	Triton X-100	Tween 20
C. sorokiniana	50	0.5	50	50	50	0.1	0.1	0.1	0.1	50
S. obliquus	50	0.1	50	10	50	0.1	5	0.1	2.5	50

Surfactants showing acceptable properties with respect to each individual criterion were indicated with a '+' in Table 2. Considering all properties together, the poloxameric surfactants are the best candidates investigated for the operation of the liquid foam-bed photobioreactor, due to their good foaming properties, resistance to biodegradation, moderate algae partitioning, and low toxicity. The most important limitation of these surfactants lies in the fact that they have low P_x^{foam} values (i.e. pa~~o~~rtioning towards the foam). By minimizing the amount of bulk liquid in an operational foam-bed photobioreactor, the absolute amount of algae in the relatively dark bulk liquid can be minimized and the majority of the microalgae still reside in the foam phase. Furthermore, the algae partitioning towards the foam formed by poloxamers can be further improved in the foam-bed photobioreactor by increased surfactant concentration,[41] increased aeration rates,[14] and reduced bubble sizes bubbles.[39, 52, 53] Alternatively, the microalgae-enriched bulk liquid phase can be recirculated and distributed on the top of the foam column. From the two tested poloxamers, Pluronic F68 was selected as the best surfactant since it performed slightly better in terms of foaming, algae partitioning and toxicity.

The Foam-bed photobioreactor is a promising photobioreactor concept with several advantages over liquid-phase based cultivation systems. Foam-bed photobioreactors can offer reduced light absorption path and increased mass transfer rates compared to traditional photobioreactors. Surfactant selection is a key factor in the success of the operation. Pluronic F68 appeared to be a surfactant which can allow for long-term operation of foam-bed photobioreactors. High biomass densities and reduced gas flow rates and pressured drops in foam-bed photobioreactors will then contribute to a reduction in biomass production costs.

Table 2. Summary of the surfactant selection experiments. The "+" indicates that a certain surfactant has acceptable properties regarding the corresponding criterion. Foaming properties: foam volume half-life time (A), liquid holdup in the foam (B), bubble size of the foam (C) and surfactant stability (D).

	Foaming properties				Algae partitioning	Resistance to biodegradability	Toxicity
	A	B	C	D			
BSA	+	+	+	-	+	-	+
CTAB	-	-	+	+	+	+	-
Pluronic F68	+	+	+	+	-	+	+
Pluronic P84	+	+	+	+	-	+	+
Saponin	-	-	-	+	+	-	+
SDS	-	+	+	+	-	-	-
Tergitol NP9	+	-	+	+	-	-	-
Tergitol TMN6	-	+	+	+	-	-	-
Triton X-100	+	-	+	+	+	+	-
Tween 20	-	-	+	+	-	-	+

Conclusions

This project was undertaken to select the best foam stabilising agent to be applied in a liquid foam-bed photobioreactor. First a pre-selection was made of 10 potential candidates which were tested in terms of their foaming properties, biodegradability, microalgae partitioning towards the foam phase, and toxicity. According to the experimentally obtained data, poloxameric surfactants (Pluronic F68 and Pluronic P84) exhibited the best properties and Pluronic F68 was selected as the best surfactant because of its slightly better performance. Pluronic F68 showed constant foamability during repetitive foaming cycles and at 5 CMC concentration it formed foams with intermediate stability (54 minutes half-life), high liquid holdup (8-% at 400 mL min^{-1} superficial gas velocity), and small bubble size (radius of 0.17 mm) which are optimal for the operation of the liquid foam-bed photobioreactor. Besides, it is hardly biodegradable by the bacteria naturally-associated to microalgae, therefore the surfactant concentration in the reactor is not reduced due to bacterial consumption. The relatively low microalgae partitioning towards the foam phase formed by Pluronic F68 (67-% for *C. sorokiniana* and 47-% for *S. obliquus*) can be compensated by optimizing operation and design of a liquid foam-bed photobioreactor. For example, foam-bed photobioreactor systems where the microalgae-enriched liquid layer is recirculated over the foam phase could result in an economically feasible algae production system. –Finally, growth inhibition was not observed for any of the microalgal strains tested until 50 CMC. Thus, Pluronic F68 is a promising, new foam stabilising agent for a liquid foam-bed photobioreactor.

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The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Supplementary material

Modified Breuer's medium

The recipe used in this study contains KNO_3 2.55 g L^{-1}, Na_2SO_4 0.15 g L^{-1}, $MgSO_4 \cdot 7H_2O$ 0.22 g L^{-1}, $CaCl_2 \cdot 2H_2O$ 0.11 g L^{-1}, K_2HPO_4 0.645 g L^{-1}, Na_2-EDTA$\cdot 2H_2O$ 44.64 mg L^{-1}, $MnCl_2 \cdot 4H_20$ 5.72 mg L^{-1}, $ZnSO_4 \cdot 7H_2O$ 1.8 mg L^{-1}, $CoCl_2 \cdot 6H_2O$ 0.423 mg L^{-1}, $CuSO_4.5H_2O$ 0.495 mg L^{-1}, $Na_2MoO_4 \cdot 2H_2O$ 0.0378mg $L^{-1}$and NaFeEDTA 15.3 mg L^{-1}. The pH of the media was set to 7.0.

Modified M8a medium (algae partitioning)

The media contained the following nutrients: urea 3.60 g L^{-1}, KH_2PO_4 1.07 g L^{-1}, $Na_2HPO_4.2H_2O$ 0.37 g L^{-1}, $MgSO_4 \cdot 7H_2O$ 1.2 g L^{-1}, $CaCl_2 \cdot 2H_2O$ 39 mg L^{-1}, EDTA ferric sodium salt 347.8 mg L^{-1}, $Na_2EDTA \cdot 2H_2O$ 111.67 mg L^{-1}, H_3BO_3 0.185 mg L^{-1}, $MnCl_2 \cdot 4H_2O$ 38.9 mg L^{-1}, $ZnSO_4 \cdot 7H_2O$ 9.6 mg L^{-1}, $CuSO_4 \cdot 5H_2O$ 5.49 L^{-1}. After the addition of all nutrients, the pH was adjusted with NaOH to pH 6.7.

Table S1. Summary of the foam stabilizing agents used in this study. P.I. stands for the online product information sheets. The superscript 'a' indicates the CMC value used for the foaming and partitioning experiments, while 'b' indicates the values used for the biodegradability and toxicity experiments.

Foam stabilizing agent	**Source details**	**Type**	**CMC $[g L^{-1}]$**	**Ref. CMC**	**Chemical composition**
BSA	Sigma, A7030	Non-catalytic protein, anionic character	0.03	[35], 25°C	Bovine Serum Albumin derived from cows
CTAB	Sigma H5882	Cationic	0.335 [a] 0.346 [b]	Sigma P.I., 20-25°C [a], minimum given value Sigma P.I., 20-25°C[b], middle of given range	Hexadecyltrimethylammonium bromide
Pluronic F68	Panreac A1288	Non-ionic	0.334	Sigma Aldrich P.I.	Triblock copolymers of ethylene and propylene oxide
Pluronic P84	Sigma 713538	Non-ionic	0.298	[54]	Triblock copolymers of ethylene and propylene oxide
Saponin	Sigma 84510	Non-ionic	0.05	Sigma P.I. ,middle of given range	Glycoside+ Triterpene
SDS	Sigma L3771	Anionic	2.451	Sigma P.I., middle of given range 20-25°C	Sodium dodecyl sulfate
Tergitol NP9	Sigma 521078	Non-ionic	0.06	DOW P.I., 25°C	Nonylphenolethoxylate
Tergitol TMN6	Sigma 60828	Non-ionic	0.8	DOW P.I., 25°C	Branched secondary alcohol ethoxylate
Triton X-100	Panreac A1388	Non-ionic	0.155 [a] 0.194[b]	[55] [a] ApplichemP.I.[b]	Octyl phenol ethoxylate
Tween 20	Sigma, P2287[a] Sigma, P1379 [b]	Non-ionic	0.099[a] 0.06[b]	[56], 21 °C [a] [57], room temperature [b]	Polyoxyethylene (20) sorbitanmonolaurate

Mass balances microalgae partitioning

Mass balances for the microalgae cells over the foam-bed system were set up in order to explain in detail the microalgae partitioning coefficient and the factors influencing that. These equations correspond to the experimental setup of the microalgae partitioning experiments performed. A scheme of the experimental setup for microalgae partitioning is presented in Figure S1.

Equation S1 describes that the cells present in the initial liquid prior to foam formation will be distributed over the bulk liquid and the liquid fraction of the foam after foam formation. Equation S2 explains that also the initial liquid volume will be distributed over the bulk liquid phase and the foam phase after foam formation. The notation corresponding to these equations is presented in Table S2.

The partition coefficient (Equation S3) results in exactly 1 when there is no preference for the algal cells for either the foam phase or bulk liquid phase. P_x^{foam} values above, or below 1, give a relative indication of the level of preference of algal cells for either phase.

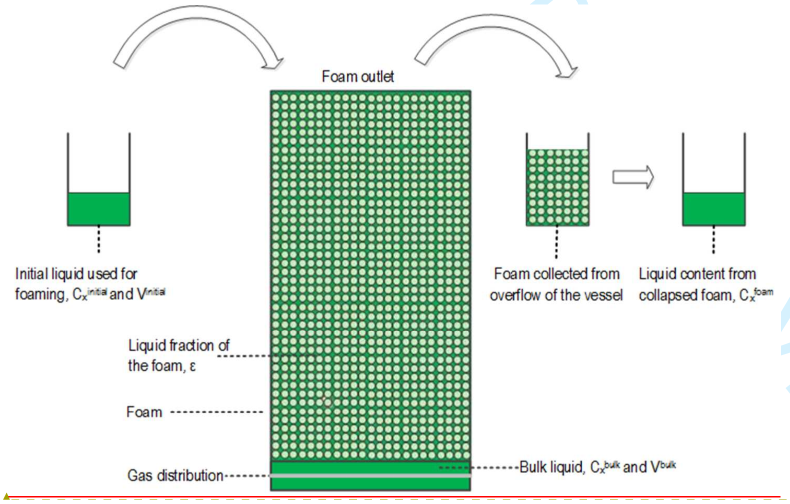

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Figure S1. Scheme of the experimental procedure for microalgae partitioning measurements.

$$V^{initial} \cdot C_x^{initial} = C_x^{bulk}(t) \cdot V^{bulk}(t) + V^{total}(t) \cdot \varepsilon_{avg}(t) \cdot C_{xavg}^{foam}(t) \qquad \text{Equation S1}$$

$$V^{initial} = V^{bulk}(t) + V^{total}(t) \cdot \varepsilon_{avg}(t) \qquad \text{Equation S2}$$

$$P_x^{foam} = \frac{C_x^{foam}(t=t_{test})}{C_x^{initial}} \qquad \text{Equation S3}$$

The algae partitioning experiments were done in a batch system and therefore the bulk liquid volume, bulk liquid algae concentration and bulk liquid surfactant concentration might change in time with increasing foam volume. In addition, the liquid fraction of foams decreases in height due to gravitational drainage in the foam. Consequently, in the equations the average liquid fraction along the foam height has to be considered. The biomass concentration in the foam is therefore also not homogenously distributed in the different foam fractions.

The dynamics of the systems discussed above did not influence our measurements. We collected less than 20% of the total liquid volume which was initially added and no trends were observed between the different foam fractions collected in time. Changes in the partitioning apparently were not observable within the experimental time and foam volumes sampled.

To avoid any influences of experimental conditions on P_x^{foam}, the same protocol was used for each measurement. This includes a fixed foam volume collected, a fixed total foam volume and therefore fixed foam formation time, a fixed gas flow rate, initial liquid volume, initial algae concentration, and surfactant concentration. In this way the different surfactants can be appropriately compared in terms of algal partitioning as defined with Equation S3.

Table S2. Notation related to the mass balances.

Symbol	Explanation
ε	local liquid fraction in the foam, thus the liquid volume incorporated in the foam divided by the foam volume
ε_{avg}	Average liquid fraction in the foam
$C_x^{initial}$	biomass concentration in the initial solution, before foam formation
C_x^{bulk}	biomass concentration in the bulk liquid phase
C_x^{foam}	local biomass concentration in the liquid fraction of the foam
$C_{x,avg}^{foam}$	average biomass concentration in the liquid fraction of the foam
V^{bulk}	bulk liquid volume (liquid layer underneath the foam)
V^{total}	total foam volume
$V^{initial}$	total liquid volume of the foaming solution, measured prior to foam formation