DEVELOPMENT OF THE MICROBIOLOGICAL POPULATION IN WATER MISCIBLE METAL WORKING FLUIDS

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ABSTRACT
The deterioration of metal working fluids (MWFs) due to the microbial colonization and degradation is a considerable economic factor in the metal working industry. Microorganisms (MO) are able to metabolize almost all components of MWFs and thus lead to a loss of its function by the reduction or depletion of additives. Microbial growth cannot be avoided completely, although various methods exist to reduce the bacterial load in MWFs. This paper presents a study on the colonization of MWFs by bacteria and fungi in an industrial environment. The cooling lubricants have been periodically examined based on biological and chemical methods. The level of the total bacterial load in the lubricant is considered as well as the composition of the species community and its development over the evaluation period. With regard to the increasing relevance of environment friendly processes, a conventional mineral oil based MWF has been compared to a product based on renewable resources.
KEY WORDS: lubrication, cutting fluids, microbiological deterioration

TABLE OF ABBREVATIONS

MWF	metal working fluid
MO	microorganism
CFU	colony forming units
ATP	adenosine triphosphate

1.- INTRODUCTION
In modern metal working industry, metal working fluids (MWFs) play an essential role for a wide range of machining processes. As primary tasks of MWFs, the removal of heat from the contact zone and the reduction of friction between tool and workpiece are to be mentioned. Especially in grinding processes, a suitable MWF-supply is necessary for removing chips and swarf from the cutting zone [1]. For the maintenance of water miscible MWFs, microbial colonization and biodeterioration is of great importance. MWF-cycles are more or less open systems with contact to the surrounding environment. Favourable temperatures in machine tools and storage tanks as well as the permanent availability of water and nutrients make MWF-systems to a suitable habitat for microorganisms (MO). Modern MWFs contain manifold biodegradable substances, which are potential nutrient sources for a wide range of bacteria and fungi. High rates of microbial degradation could be obtained for e.g. fatty acid amides, fatty acids, fatty alcohol ethoxylates and fatty ethanol amide [2]. The influence of biological contamination of MWFs on the technical performance is schematically outlined in Figure 1. Whenever a bacterium enters a new ecological system and comes into contact with digestible nutrient sources, the metabolism is activated to enable the uptake of these nutrients and subsequently the organism's reproduction. This start-up phase is referred to as latency. In the following, oil and additives of the MWF are metabolised with increasing performance.

The decreasing additive concentration is expected to lead to a loss of technical performance, e.g. increasing tool wear, surface roughness or cutting forces. The influence of the biological contamination on the technical performance of cutting fluids is a key issue of current MWF-research [3]. Further consequences of heavy microbial load are fouling and smell, up to mechanical problems in the MWF-cycle due to clogging of filters, pipes etc. by biofilms. Therefore, the latency phase should be enlarged as much as possible to increase the MWF-life time.

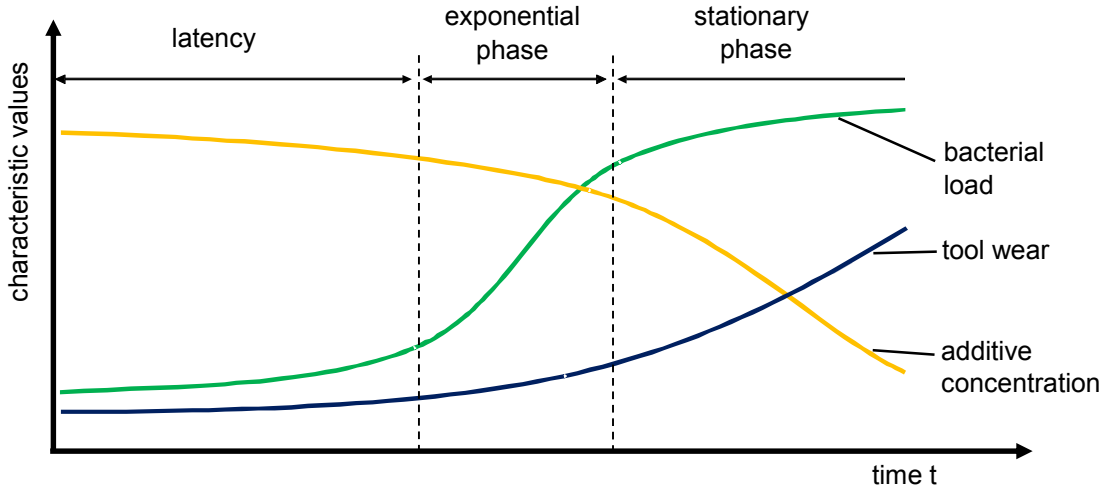

Figure 1: Schematically influence of biological colonization of a water miscible MWF

The microbiology of MWFs has been subject of many research activities and is investigated since the 1940s [4]. Several authors have located and identified more than hundred different species of bacteria and fungi in MWFs [5]. In general, much more bacterial than fungal species were isolated from water miscible MWFs. The most relevant bacterial MWF-colonisers belong to the *Pseudomonas* genus, while *Fusarium* is the mainly represented genus among fungi [6].

Various methods exist to reduce microbial colonization of MWFs. Among the primarily utilized biocides (bactericides and fungicides) the following are to be named: formaldehyde releasers (N- and O-formals), heterocyclic compounds and further biocides (penoxyalcohols, carbamate, quaternary ammonium compounds) [7]. However, biocides reveal disadvantages such as being hazardous to humans and environment, selective impact, bacterial resistances (genetic adaption) and limited diffusion through biofilms [8]. The working mechanism of biocides is mainly based on chemical effects. Physical working methods such as UV or gamma radiation, ultrasound, thermal and ozone treatment influencing the bacterial load are also investigated. Due to some substantial disadvantages (selectivity, damage to emulsion stability, high costs, etc.), the application in the industrial field could still not be realized [9].

The existing approaches only allow a deceleration of bacterial colonisation but no complete prevention. For an efficient application of MWFs in machining processes, a reliable and precise monitoring is essential. Therefore, several tests exist. Some are widely distributed in industry, while others are mainly employed in research contexts. The pH-value is easy measurable and indicates organic activity due to the release of protons by MO. A low pH-value leads to corrosion and emulsion instability. The MWF-concentration is another important parameter. By adjusting the concentration, a balance between low operating costs and sufficient MWF-performance has to be found. To avoid hazards to health and environment by the formation of nitrosamines,

the monitoring of the nitrate and nitrite concentrations is mandatory. Furthermore, a rising nitrate level indicates high bacterial activity. The practical benefit of these methods is limited due to the fact that none of them enables the precise quantification of the bacterial load.

To evaluate the number of living bacteria in MWFs, various strategies were established. To determine the colony forming units (CFU) per millilitre by incubation, a defined volume of MWF is spread on a culture medium and incubated for few days. Subsequently, the grown colonies are counted with the assumption that each colony came up from a single bacterial cell. Thus, the colony forming units per millilitre can be quantified. This method is labour-intensive and requires biological laboratory technology, but therefore it is relatively precise. Disadvantages are the bacterial selectivity regarding parameters such as substrate, incubation time or exposure to oxygen. Furthermore, this method takes several days to receive the test result [10]. The estimation of CFU by dip slides is much easier but also more imprecisely. Dip slides are plastic carriers with culture medium, which are dipped in the MWF. Accordingly, the volume of the liquid and thus the number of incubated cells depends on the viscosity of the MWF. Moreover, the colonies are not counted individually; the growth intensity is just estimated by visual impression in comparison to given reference values. The incubation takes usually between 24 and 72 hours and is limited by substrate selectivity [11]. Although the determination of the bacterial load via biological reproduction of living cells reveals expedient data, the named disadvantages inhibit these methods from broad industrial applications.

A new approach to determine the bacterial load is the estimation via adenosine triphosphate (ATP), the universal biological energy carrier. With a multistage test, the present ATP is utilized to induce the emission of light via a luciferin/luciferase-complex. The present number of bacteria in the MWF is determined in relation to the emitted light [12]. An uncertainty of this technique lies in the varying amount of ATP in living organisms. While few very active bacteria provide a high amount of ATP, many “sleeping” bacteria can hardly be detected. However, on closer consideration this characteristic turns to a practicable advantage, due to the fact that especially the active MO are relevant for the degradation of MWFs. The transfer of this technique into industrial application is limited due to the complexity of the measurement procedure.

It is apparent, that current MWF-monitoring methods only reveal an incomplete description of the MWF-cycle as an ecological system including an active and dynamic species community. Neither the presence of individual species nor their effect of or correlation with the chemical composition of MWFs are considered by conventional monitoring. Therefore, the investigation of the bacterial colonization of MWFs is a subject which requires further comprehensive research work. A contribution to this issue is given by this paper. Researching the microbial deterioration of MWFs requires highly interdisciplinary collaboration, primarily including production engineering, chemistry, and biology. The structure and constitution of the present species and their progress in the course of time is of particular interest. This paper presents a study about the colonization of MWFs by MO.

2.- METHODICAL APPROACH AND EXPERIMENTAL SETUP

Biological screenings of two different cooling lubricants in service in a machine tool were conducted weekly to establish an overview about the species diversity in the ecosystem “MWF” and its development over time. One MWF is a conventional

product based on mineral oil while the other lubricant is a mineral oil free MWF made from renewable resources. They were employed in various metal cutting processes. To accelerate the biological deterioration, the formulation of the MWFs contained no biocides. The study lasted for a period of 100 days (mineral oil free) and 70 days (conventional), respectively. The initial MWF concentration was set up to 5 %. No concentrate was added within the duration of the experiment, just water to keep the volume level constant. The bacterial load was quantified by determining the CFU through incubation on a culture medium and via ATP. Moreover, the present species were identified each week to monitor as well the development of the species community.
The biological analysis was carried out once a week. The samples were diluted using a weak detergent solution. 50 µl of each dilution were plated on different culture media for the enrichment of bacteria and fungi, respectively, and incubated at 25 °C for 3-7 days. Subsequently, colony forming units were counted and microbiological contamination was calculated as CFU/ml. The species were determined by MALDI-ToF with using a Microflex mass spectrometer (Bruker Daltonik, Bremen, Germany). The determination of the bacterial load via ATP test was conducted to achieve further data and enable a comparison of the results.

3.- RESULTS AND DISCUSSION

The results of this study distinctly expose the substantial relevance of microbiological colonisation for water miscible MWFs for the lubricant's maintenance. Despite the conventional conservation with biocides, both MWFs were affected by a severe bacterial load. After four weeks of exponential colonization, the MO reached a level of about 10^7 CFU/ml in both lubricants. Interestingly, the two methods measuring the bacterial load revealed varying data for low bacterial contamination below 10^5 CFU/ml. While the incubation test indicated no CFU in the very beginning, the ATP-test displayed 10^4 CFU/ml in each freshly prepared emulsion, indicating a slight influence of the MWF itself on the ATP-test results. Furthermore, significant differences between the conventional and the mineral oil free MWF could be observed regarding the composition of the species community. The conventional product contained a considerable higher species diversity than the mineral oil free lubricant. Only few bacteria such as the pioneer species *Pseudomonas mosselii* were detected in both MWFs.
Conventional MWF. Figure 2 illustrates the development of the bacterial load on a logarithmical scale and the number of identified species. The level of active MO increased very quickly to about 10^7 CFU/ml within two weeks. The bacterial load stayed between 10^7 and 10^8 CFU/ml in the remaining time.

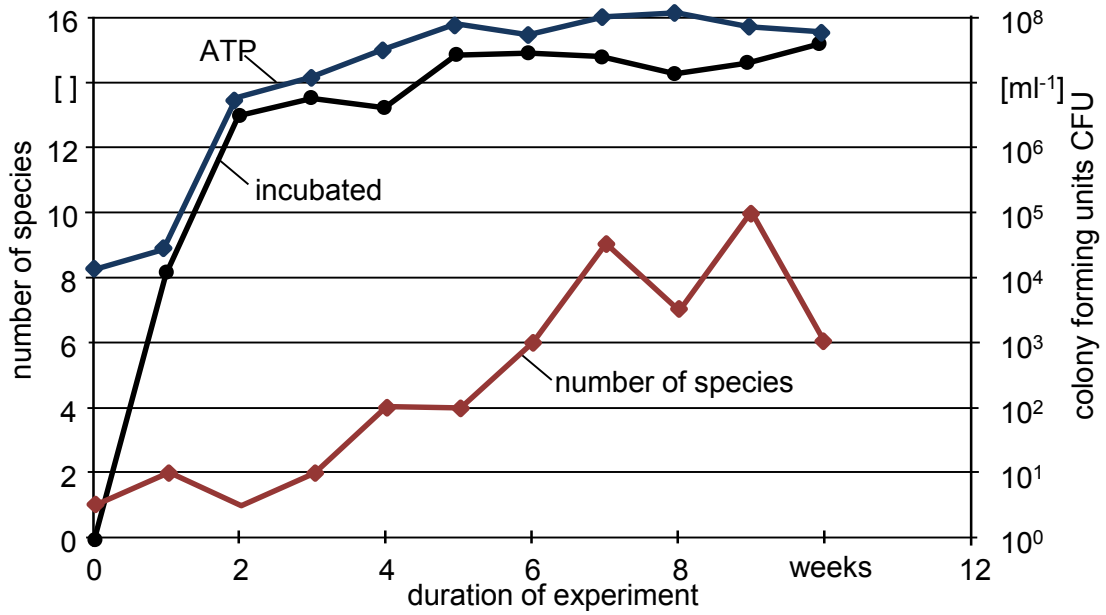

Figure 2: The bacterial load and the number of present species over time of the conventional MWF

Interestingly, there is a different trend regarding the bacterial load and the number of species depicted by the lower line. While the bacterial load is rapidly increasing within the first weeks, the number of species takes more time to grow significantly. In other words, the considerable reproduction was performed by only few species of bacteria, what is apparent in Figure 3. The diagram illustrates the composition of the species community for the entire duration of the study. As it can be seen, a dominant species in the beginning was *Pseudomonas mosselii*.

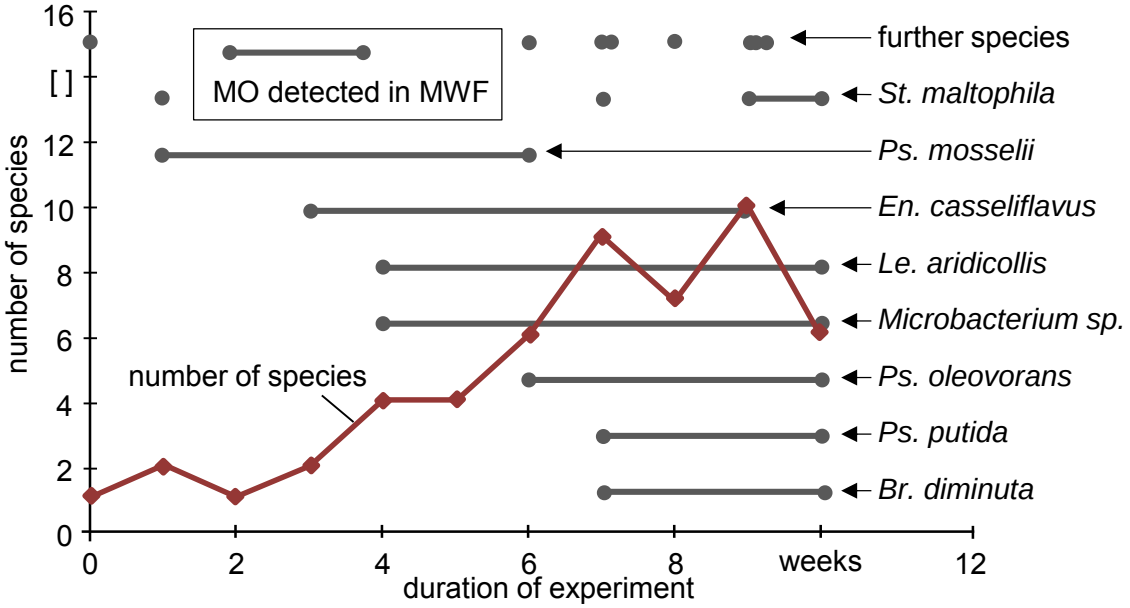

Figure 3: The development of the species diversity and number of present species over time of the conventional MWF

This pioneer species were able to enter the ecological system in its initial state. The degradation of MWF-components and the according release of metabolic products increased the spectrum of available substances and allowed more species to start

metabolic activity. The number of species was continuously increasing. In course of the experiment, the species community shifted due to the available nutrients. Pioneer species such as *Pseudomonas mosselii* vanished due to the selection pressure of the rising colonization by *Enterococcus casseliflavus*, *Leucobacter aridicollis* and several further MO, compare Figure 3. No species was detected over the entire duration of the study. After 7 weeks, a broad biodiversity had established, revealing the continuous appearance of several species such as *Brevundimonas diminuta*.
Mineral oil free MWF. Figure 4 depicts the bacterial colonization of the mineral oil free MWF. The bacterial load was rapidly increasing within the first four weeks and remained later on a level of 10^7 CFU/ml, comparable with the conventional product. Again, the two graphs showing the concentration of living cells reveal a large deviation in the beginning of the experiment.

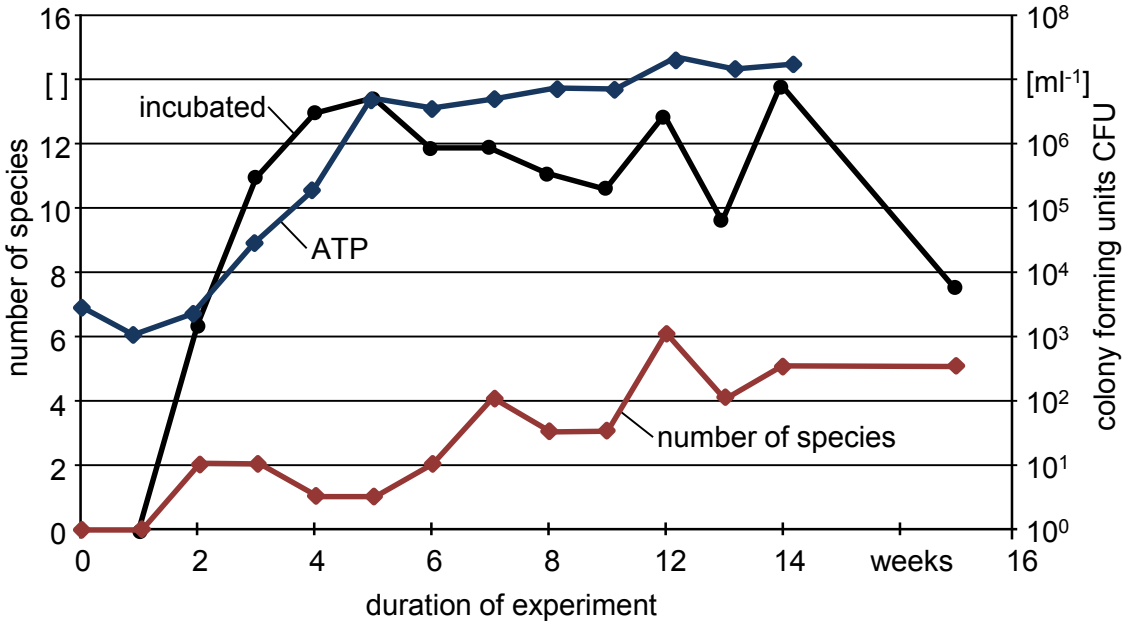

Figure 4: The bacterial load and the number of present species over time of the mineral oil free MWF

Compared to the conventional product, the number of different species increased relatively slow and the maximum of detected species was lower, too. Only a few species were responsible for the enormous microbial activity in the first weeks. A reason for the smaller spectrum of species could be located in the MWF-composition. This product based on renewable resources is formulated with considerably less components than the conventional MWF. Fewer available nutrients lead to fewer active species. The nonetheless high suitability of the mineral oil free MWF can be inferred from the high bacterial cell counts of 10^7 CFU/ml after four weeks. The collective of present species in the mineral oil free MWF was as well changing over time as it can be seen in Figure 5.
Although fewer species at a time were found, the overall number of detected species is comparable to the conventional product. Important pioneer species in the mineral oil free MWF were *Pseudomonas mosselii* and *Pseudomonas oleovorans*, while *Brevundimonas diminuta* and representatives of the genus *Providencia* were relevant MO in the last weeks.

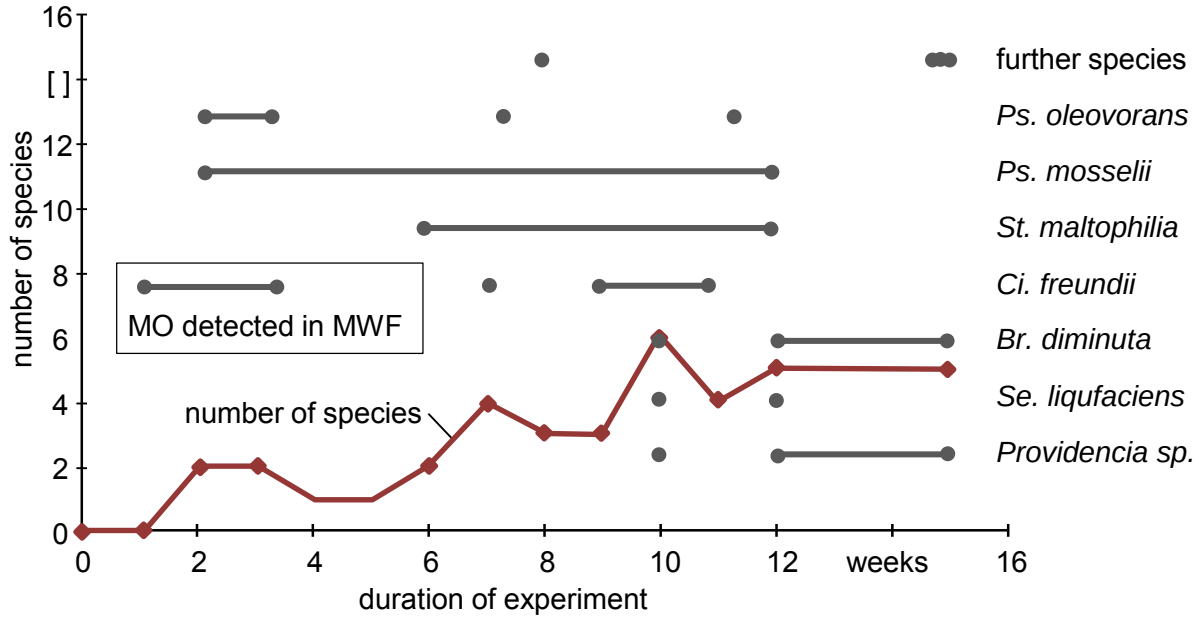

Figure 5: The development of the species diversity and number of present species over time of the mineral oil free MWF

4.- CONCLUSION

The microbial colonization plays a significant role in the degradation of water miscible MWFs. Independent from the basic MWF-substance, in freshly prepared water miscible MWF, the bacterial load may reach 10^7 to 10^8 CFU/ml within a few weeks. Significant differences between the conventional and the mineral oil free MWF were particularly found regarding the biodiversity. In the conventional product, more species could live together in a community probably due to a wider range of available substances. The turnover of the species community could be observed in both cases. *Pseudomonas mosselii* can be emphasized as pioneer species while *Brevundimonas diminuta* appeared in both MWFs in the last evaluated weeks. With four species in the conventional and three species in the mineral oil free product *Pseudomonas* was the most represented genus. The only detected fungus was *Fusarium solani*, which was isolated once in the conventional MWF in the sixth week. The results of the colony forming units of the ATP- and the incubation method showed in both experiments a large deviation in the range of low bacterial contamination beneath 10^5 CFU/ml.

The necessity of further research work concerning the microbiology of MWF-systems is given evidence by this study. For an efficient monitoring of MWFs the understanding of cause-and-effect relationships between MO and the environmental conditions in the MWF-cycle is an essential requirement. Further studies are planned to investigate the influence of various individual factors on the development of the species community. These parameters comprise the availability of individual MWF-components and biocides, the presence or absence of oxygen, the MWF-concentration or the existence of surfaces in large quantities provided by metal chips or grinding swarf. Moreover, the improvement of monitoring methods regarding data quality and measurement frequency is mandatory for the development of more efficient and reliable maintenance systems for MWFs.

The extensive investigation of the MWF-microbiology enables the possibility to investigate MO revealing positive characteristics for an application in MWF-systems. A feasible approach is the production of essential substances for MWF by MO, which

are either directly dispersed in the applied MWF or generated in a bioreactor parallel connected to the MWF-system and supplied according to the current demand [13]. Another possible way is the direct application of MO in MWFs to substitute individual components such as lubricating additives [14].

7.- REFERENCES

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