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Effect of biomass concentration on the performance of a MBR system: Studies of microbial communities

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ABSTRACT

The performance of a bench-scale submerged membrane bioreactor (MBR) equipped with ultrafiltration membranes (Zenon®) was investigated for the treatment of domestic wastewater under different mixed liquor suspended solid (MLSS) concentration. The results showed the capacity of the MBR systems to remove high concentration in organic matter (COD and BOD_5) in order to study the influence of the removal in organic matter enzymatic activities (acid and alkaline phosphatases, glucasidase, protease, esterase and dehydrogenase); and biodiversity of the bacterial community in the sludge was measured. Cluster analysis of the temperature gradient gel electrophoresis profiles demonstrated that the community composition varied significantly in the different experiments. Depending on MLSS and temperature, the bacterial community experienced sequential shifts as the biomass accumulated, as shown by the evolution of the population profiles through time as volatile suspended solids concentration.

Keywords: Membrane bioreactor; Sewage; Enzymatic activities; Biodiversity

1. Introduction

Although conventional activated sludge processes have established their efficiency in removing contamination from sewage waters, technical developments nevertheless require new technologies better adapted to the elimination of contaminants, thus enabling the effluent to reach an acceptable quality for the direct re-use of the wastewater. The adaptability of membrane bioreactor (MBR) systems enable the possibility of the latter to adjust to an activated sludge previously present, thereby increasing the volume loading rate of the influent and/or the load that current treatment plants accommodate [1].

The MBR technology is as suitable for the treatment of domestic wastewater as it is for industrial wastewater [2]. Due to the high quality of the effluent and the exceptional compactness of the plants, the latter is adapted for conditions where the implementation in protected environments and treatment of complex industrial effluents need to operate with an aged activated sludge.

Microbial communities developed in MBR-based treatment systems are analogous to those present in conventional activated sludge. However, the microorganisms are exposed to completely different conditions, mainly due to the biomass recycling in MBR, which leads to changes in the biology of the system. Bacteria are subjected to a substrate-limited environment, which induces low growth rates and reduces the respiratory

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potential of the microorganisms, hence, influencing the activities and species composition of the sludge community [3]. Measuring biomass concentration and enzymatic activities is essential to achieve the biological characterization of a complex microbial microcosm, like the case of an activated sludge [4]. These enzymatic activities give an estimation of the active biomass, the elimination of organic matter, N and P, and are also used as indicators of the fate of specific populations. Some of the most important enzymatic activities during the biological treatment of wastewater are phosphatase, glucosidase, protease, and esterase [5].

The aim of the research was to evaluate how the different mixed liquor suspended solid (MLSS) concentrations affect the quality of the permeate and the influence of the MLSS in the enzymatic activities (acid and alkaline phosphatases, glucasidase, protease, esterase and dehydrogenase) and in the biodiversity of the bacterial community in the MBR.

2. Materials and methods

2.1. Pilot-plant membrane bioreactor

The membrane (Zenon®) is composed of a polymer of polvinyldifluoride (PVDF), containing an average pore size of 0.04 μm. The membrane is neutrally charged and hydrophilic, and, to prevent fouling, it is periodically backwashed. The membrane configuration is from the outside to the inside of the hollow-fibre; the filtrate passes to the interior of the fibre, leaving what is retained on the outside. In this way, the effluent can be treated with higher suspended solid (SS) concentrations.

The main components of the system (Fig. 1) are: the 224 L bioreactor, a control panel, a pressure pump, an air pump to generate a tangential aeration current to the fibre in the membrane in order to avoid fouling, and a 25 L backwash tank. The filtrate and backwash processes are controlled directly from the control panel. The system also contains an air compressor to supply the air needed to maintain the oxic conditions of the biological aerobic processes.

The pilot plant is located in the wastewater treatment plant of the city of Granada (Puente de los Vados, Granada, Spain). The influent used for the pilot plant came from the primary settling tank of the wastewater-treatment plant; the feed/input was controlled by a pressure pump for each membrane located at the outlet. The system is equipped with a level control so that the membranes are always covered with water, preventing the membranes from drying or ripping. Moreover, we have placed a level indicator to control the pressure pump.

2.2. Experimental procedure

The influent in the bioreactor was controlled by a level indicator connected to a feed pump. This system is controlled such that the membranes were always covered by the mixed liquor.

Once the membranes were submerged, the aspiration of the pressure pump forced the liquid in the bioreactor to pass through the ultrafiltration membrane leaving the solids retained of the biological reactor, thereby increasing the MLSS concentration gradually. When the desired concentration was achieved, the corresponding sludge

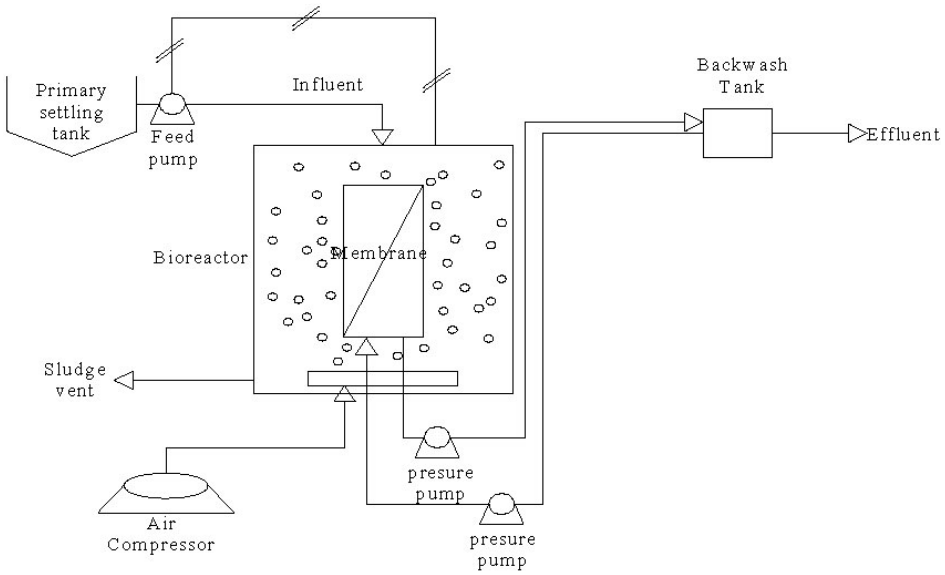

Fig. 1. Diagram of the MBR pilot-scale plant used in the study.

vents were opened accordingly to maintain the specific conditions in the reactor. The membranes were continuously aerated with tangential air currents to avoid any organic or inorganic solids from settling on their surface. A compressor provided the adequate aerobic conditions for the process. Dissolved oxygen (DO) was monitored and controlled using a oxygen electrode (Crisol OXI 320). The pressure pump inverted the flow regularly and sent the treated water into the backwash tank towards the membrane. Thus, aggregates and particles were removed from the surface membrane. The running of the plant consisted of 10-min cycles: a cycle includes 9 min 45 s of filtration and a 15-s backwash cycle.

2.3. Analytical determinations

BOD_5, COD, SS and volatile suspended solids (VSS) were determined according the APHA method [6]. Weekly sampling in all experiments of the activated sludge was done to measure the total heterotrophic platable microbiota and microbial enzymatic activities. These samples were also used for DNA extraction.

Total heterotrophic platable microorganisms (THPM) counts were conducted during six consecutive weeks in the experiments. The number of THPM was measured by standard serial dilution and plating on tryptic soy agar (TSA; Difco). Plates were incubated at both 22°C (48 h) and 37°C (24 h) before colonies were counted.

Evolution of microbial enzymatic activities in the bioreactor — Phosphatase and glucosidases determination was done by the method of Goel et al. [7]. For both phosphatases and glucosidases, standard curves were made with known concentrations of p-nitrophenol (Sigma). Protease activity was calculated by the colorimetric method of Cadoret et al. [8]. Total esterase activity was measured by the methods of Boczar et al. [9]. Dehydrogenase activity was estimated by the method of [4].

PCR amplification of partial 16S rRNA gene, TGGE, and DNA sequencing TGGE profiling of the community in the activated sludge were conducted in the experiments on a weekly basis during the periods indicated. DNA was extracted from the activated sludge samples using the Ultra Clean Soil DNA Isolation kit (MoBio, USA). A nested approach was used for PCR amplification, as this strategy has been previously reported to avoid non-specific priming, improve amplification of species present in low numbers, and yield richer band patterns in DGGE [10]. One microliter (2–5 ng) of DNA extracted from sludge was used as template for PCR using universal primers fD1 and rD1 to amplify the 16S rRNA gene in almost its full length [11]. Subsequently, 1 µl of the first PCR product was used as a template for a nested PCR using universal primers GC-P1 and C-P2, targeting the V3 hypervariable region [12]. Final PCR products were cleaned and/or concentrated (when required) using Microcon YM cartridges. Two to five microliters (60–100 ng DNA) were loaded in each well for TGGE, using a TGGE Maxi system (Whatman-Biometra).

Portions of individual bands on silver-stained TGGE gels were picked up with sterile pipette tips, placed in 10 µl of filtered and autoclaved water, and directly used for reamplification with the appropriate primers. PCR products were purified by gel running and extraction with the Quiaex-II kit (Quiagen). DNA recovered was directly used for automated sequencing in an ABI Prism 3100 Avant Genetic Analyzer.

Cluster analysis of TGGE fingerprints band patterns generated by TGGE were compared and clustered using the Quantity One image analysis software, version 4.6.1 (Biorad). Cluster analysis was used to investigate the relationships between TGGE profiles with the software program MEGA2 [13]. The TGGE banding patterns were converted to a binary matrix by scoring bands as present (G) or absent (A). The number of differences between profiles allowed for the construction of a p-distance similarity matrix, used to conduct a Neighbor-joining cluster analysis. Bootstrap values were calculated for each dichotomy.

3. Results and discussion

Three experiment were performed (Fig. 2**)** with different MLSS concentration and with different hydraulic retention time (HRT). Fig. 2a shows the MLSS concentration in the three experiments; experiments 1 and 2 were carried out with HRT = 11.71 h and experiment 3 with HRT = 8.05 h.

Fig. 2b shows the COD and BOD_5 in the influent of the MBR, and Fig. 2c shows the COD and BOD_5 in the permeate in our study. The HRT is the variable that most affects the elimination performance of BOD_5 and a slight improvement in elimination performance of organic matter can be achieved by increasing the MLSS. However, air consumption also steps up as the MLSS concentration increases. Therefore, our data show a high efficiency in removal of COD and BOD_5 during the three experiments studied. These results agree with others previously reported [14].

Enzymatic activities are essential for the hydrolysis and mineralization of the proteins, carbohydrates, and lipids, which are part of the organic matter removed from the wastewater [15]. When the activities measured in the three experiments are compared, significantly lower values are measured during experiment 2. External factors such as temperature and pH of the sludge have a strong influence on the enzymatic activities and solubility of

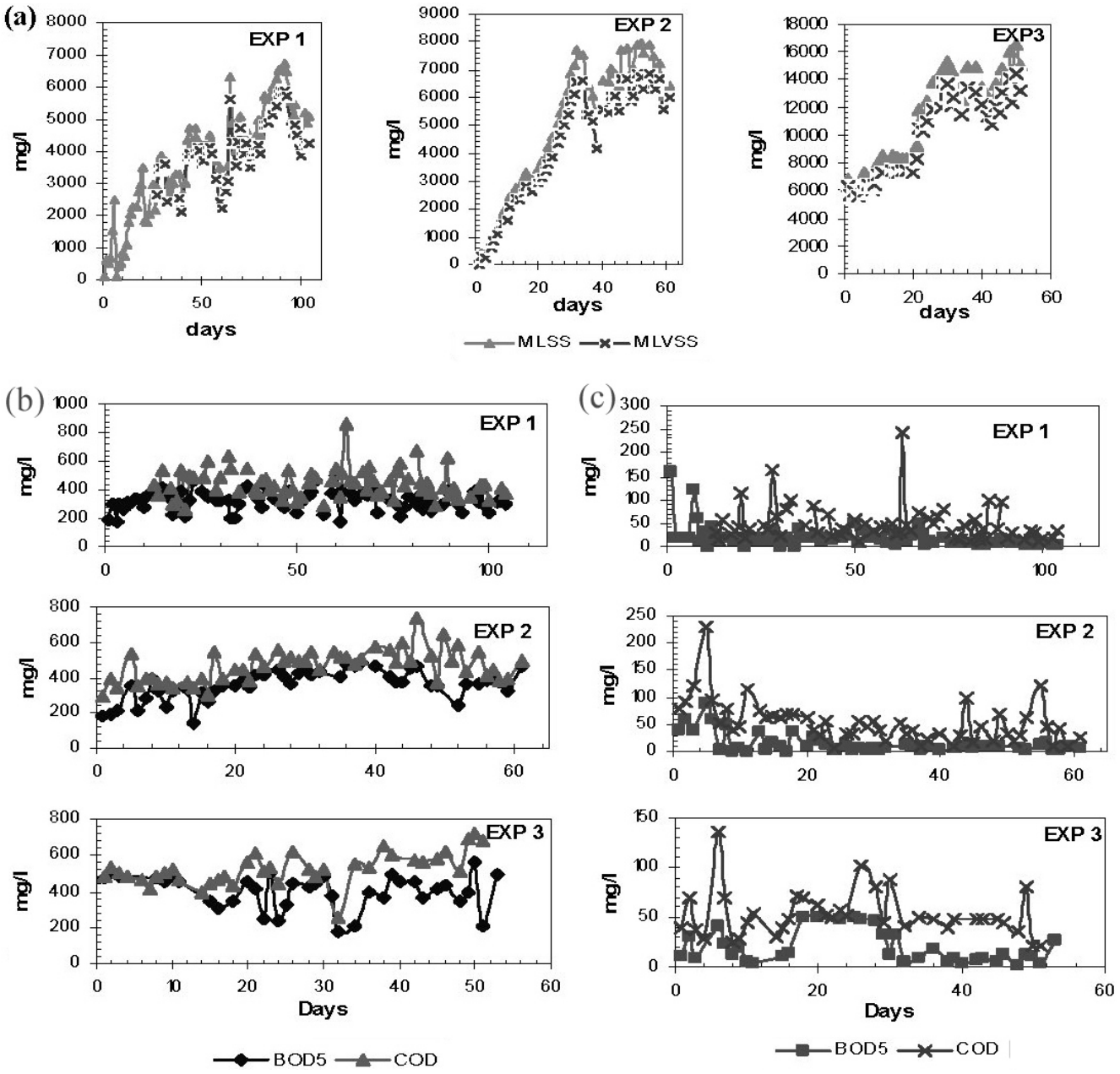

Fig. 2. (a) Concentration of MLSS and MLVSS in the MBR. (b) BOD_5 and COD in the influent of the MBR. (c) BOD_5 and COD in the permeate of the MBR.

sludge in the bioreactor [16]. In experiments 2 and 3, carried out during autumn and winter, the temperature of the sludge was 5–15°C and seldom reached over 10°C, while maximum enzymatic activities were recorded at temperatures of 13–23°C (Table 1).

The diversity of bacteria in the activated sludge from the MBR was also studied by a culture-independent approach based on TGGE, which generates profiles of the dominant populations in complex communities in the environment. This technique has been extensively applied in recent years to the study of a variety of different bacterial ecosystems, including sludge and biofilms from WWTPs [17,18]. We found that the behavior of the community responsible for the treatment of real domestic wastewater was analogous to what has been described for bacterial consortia growing under stringent nutrient limitation on synthetic wastes [3,19] and gray water [20]. TGGE fingerprints generally clustered following the time course, displaying the effect of the accumulation of MLVSS in the bioreactor on the species composition of the sludge (Fig. 3), which induced a gradual shifting of the dominant members of the community. Several bands were

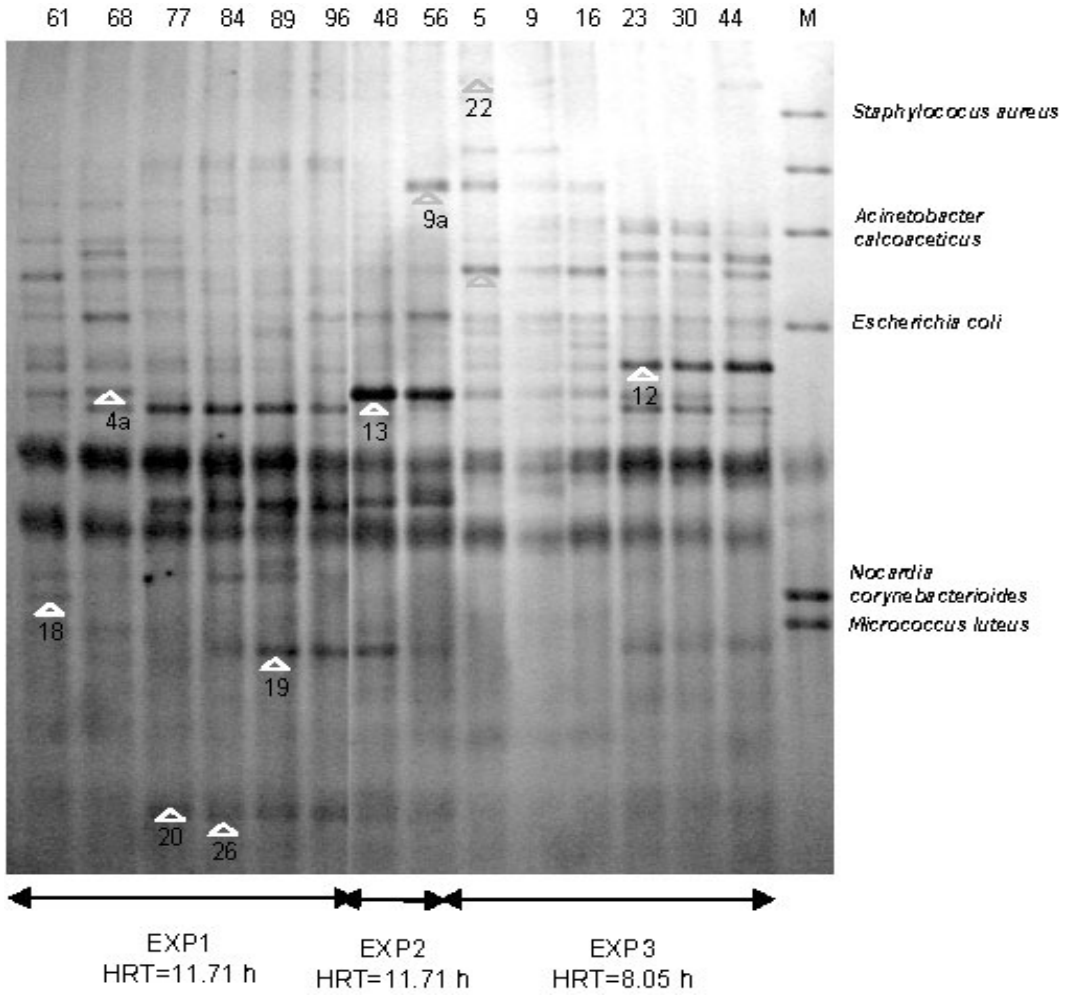

Fig. 3. TGGE fingerprinting of activated sludge samples taken from MBR. Profiles are based n amplification and separation of the V3 hypervariable region of the 16S rRNA gene. Bands that shifted, which were successfully amplified and sequenced (Table 2), are marked with white arrowheads and labelled bands.

Table 1
Optimal parameters for maximum enzymatic activities in the MBR

Enzymatic activity	mM/min/gVSS	MLVSS (mg/l)	T^a (°C)	pH	Exp.	TRH (h)
Acid phosphatase	1702	4.8	23	64	1º	1171
Alkaline phosphatase	1710	4.8	23	64	1º	1171
α-Glucosidase	242	5.683	13	73	2º	805
Protease	12.32[a]	1.72	17	73	1º	1171
Esterase	2486	2.733	21	74	1º	1171
Deshydrogenase	33	10.25	9	75	3º	805

[a]Protease activity units expressed as Δ absorbance/min/ gSSV.

detected in the TGGE profiles, which were specific of certain stages of a particular experiment (Fig. 3).

Fig. 3 shows how in the end of each experiment there are representative bands when the MBR has been stabilized under the experimental condition studied. It can be seen that during experiments 1 and 2, this band has been affected by the temperature, MLSS concentration and also has been affected by the HRT (see Fig. 3, experiments 2 and 3).

Table 2 shows the closest phylogenetic affiliations of ten of the TGGE bands that experienced shifting through the MBR operation which were successfully reamplified

Table 2
Identification of bands in the TGGE community fingerprint of the activated sludge

Band	Closest taxonomic affiliation (class/family)	Overlap(nt)	Most Similar organisms	Access no.	Percent identity
4a	α-proteobacteria/ Caulobacteraceae	121	*Caulobacter* sp. S142	AY972383	100
			Brevundimonas sp. H2/98-FUNDUS	AJ313427	992
8a	α-proteobacteria/ Sphingomonadaceae	123	*Blastomonas* sp.	AB242676	992
			Sphingomonas sp.	AJ812013	992
9a	α-proteobacteria	123	*Sinorickettsia chlamys*	AY174894	902
			Roseobacter sp.	AY136130	886
12a	β-proteobacteria	149	*Rhodoferax* sp.	AY788978	966
			Rhodoferax fermentans	AJ289107	
13a	γ-proteobacteria/ Thiotrichaceae	112	*Thiothrix eikelboomii*	L79965	100
			Thiothrix sp.	AB166732	973
18a	Actinobacteria	127	*Microthrix parvicella*	X93044	969
		124	*Collinsella* sp.	AB064936	887
19a	Bacteria/ Nitrospiraceae	125	*Candidatus Nitrospira defluvii*	DQ059545	100
			Nitrospira cf. moscoviensis	AF155154	992
20a	Gemmatimonadetes	142	*Gemmatimonas aurantiaca*	AB072735	838
	Actinobacteria	141	*Saccharopolyspora* sp.	AF131491	823
22a	Bacteria	123	Bacterium TSA-5.4	AF240150	967
	α-proteobacteria/ Methylobacteriaceae		*Roseomonas* sp.	AY624051	951
26a	Gemmatimonadetes	159	Bacterium Ellin 5290	AY234641	893
			Gemmatimonas aurantiaca	AB072735	862

from the gel and further sequenced. Representatives of the alpha-proteobacteria (bands 4a,8a, 9a and 22a), thiothrix filamentous gamma-proteobacteria (band 13a), photo-heterotrophic beta-proteobacteria (band 12a), nitrite oxidizers of the genus *Nitrospira* (band 19a), and Gemmaatimonadetes (bands 20a and 26a) were identified.

4. Conclusions

The present study showed that the enzymatic activities and bacterial community in the sludge of a submerged MBR were affected by MLSS concentration, temperature and HRT. However, under each experimental condition assayed, the enzymatic activities and community fingerprints (TGGE) demonstrated that the bacteria adapted progressively to each condition, obtained a permeate of high quality in response of the adaptation of the microbial population.

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