Professor Navarro

B-tech level : Biological treatment of waste water and sludge

Figure 1 illustrates the water cycle in town ; in this course, we will study biological treatment applied to waste water and sludge purification, not to drinking water production.

1. Municipal waste water quality and discharge standard

MWW quality is rather constant ; this board illustrates the average chemical composition od MWW and the discharge standard (from "Pollution control acts, rules and notifications issues thereunder", Central Pollution Control Board, May, 1998)

Parameter	**unit**	**Average value**	**Discharge standard in inland surface water**
pH		7.5 to 8.5	5.5 to 9
Suspended Solids SS	mg / l	150 to 500	100
BOD_5	mgO_2/l	100 to 400	30
COD	mgO_2/l	300 to 1,000	250
NH_3	mgN/l	20 to 80	50
TKN	mgN/l	30 to 100	100
Nitrate NO_3^-	mgN/l	<1	10
P	mgP/l	10 to 25	5

2. The necessity to remove BOD, ammonia, nitrates and phosphorus from waste water

This board summarizes the origin and the toxicity linked to the presence of various chemicals in MWW.

Undesirable compound C	**Origin in MWW**	**Toxicity or inconvenient for the receiving water**
SS	Wastes	Turbidity, consumption of oxygen…
Organic carbon : BOD_5	Organic matter	Consumption of O_2 in the medium
Ammonia **Organic nitrogen**	Ammonification of urea, proteins… Proteins, urea…	Consumption of O_2 Toxic for fauna
Nitrates	Absent in MWW Present in water after nitrification of ammonia	Eutrophication
Phosphorus	Organic matter and washing powders…	

3. General considerations about biological treatment

3.1. Total removal of carbon, nitrogen and phosphorus

MWW are biodegradable :
- the ratio COD / BOD, called biodegradability ratio, is inferior to 3 ;
- pH is neutral ;
- there are nitrogen, phosphorus, and no toxic.

Bacteria will be exploited for purification of MWW. They assimilate carbon (BOD), nitrogen and phosphorus according to the mass ratio : BOD / N / P = 100 / 5 / 1.

Assimilation is the intracellular incorporation of a chemical in order to carry out anabolism.

However, the ratio BOD / N / P of a MWW presents a large excess of nitrogen and phosphorus ; it can be for example equal to 100 / 20 / 10. It means that a simple assimilation doesn't allow to respect the discharge standard.
Two other biochemical processes should be performed in order to remove enough nitrogen and phosphorus :
- nitrification, and then denitrification, for total nitrogen removal ;
- specific intracellular accumulation of phosphates, for total phosphorus removal.

3.2. The separation between biomass and treated water.

Once the metabolism is performed, the exploited microbes must be separated from treated water ; there are two techniques : attached or suspended growth.
The efficiency of these two technologies depend on the bacterial secretion of an extracellular polymer : **figure 2**.
Biofiltration is performed in one step, but is a discontinuous process : when the medium is clogged, it must be backwashed. This medium both retains SS and is the place of biological purification of MWW. **Figure 3** illustrates the structure of a biofilm.
Suspended growth is a continuous two-step process : biological purification and then settling in order to separate biomass from treated water . **Figure 4** illustrates a biofloc structure.

4. Activated sludge and suspended growth

MWW is introduced in an aerated tank : biological purification takes place and flocculating biomass grows.
A real food chain develops in this tank, responsible for purification, and including a large variety of organisms :
- bacteria
- protozoan
- metazoan.

The identification of these organisms allows to check the running of the process : some organisms are indicators of the treatment quality: **figures 5 to 14.**
Figure 15 illustrates a food chain in an aerated tank.
Figure 16 illustrates the flow sheet of an activated sludge process ; as biomass grows and circulates from the tank to the clarifier, sludge must be recirculated (in order to maintain a constant biomass concentration in the aerated tank) ; the sludge draw-off allows to remove the excess of produced biomass.

4.1. TKN and BOD removal

Organic carbon is assimilated : it provides the cell with matter and energy ; these bacteria are chemo-organo heterotrophic and aerobic ; organic matter is oxidized with O_2 and the reaction generates water and CO_2.
Ammonia and phosphorus are assimilated in different biochemical ways ; in definitive, ammonia is introduced in amino acids and proteins, phosphorus in proteins, ATP, nucleic acids…

In order to remove the nitrogen excess in MWW, nitrification of ammonia must take place in the aerated tank ; it is performed by chemo-litho autotrophic bacteria like *Nitrosomonas* and *Nitrobacter*, and generates nitrates.

4.2. Nitrates removal

Nitrification produces nitrates, which induces eutrophication of the receiving water ; then nitrates must be removed.
Chemo-organo heterotrophic bacteria carry out the denitrification : they need nitrates, but also organic matter ; oxygen inhibits the reaction : nitrates are the final acceptor of electrons and many bacteria prefer using oxygen (they can carry out both respirations).
Nitrates are transformed into N_2.

The process presents two characteristics : **figure 17**
- mixed liquor is recirculated in an upstream zone : water inlet must contain organic matter (BOD is not assimilated yet)
- this zone is anoxic : oxygen inhibits denitrification.

4..3. Phosphorus removal

The excess of phosphorus is removed by a specific metabolism, carried out by bacteria like *Acinetobacter, Moraxella*…: **figure 18**
It is a two – step process : anaerobiosis then aerobiosis ; anaerobiosis means neither nitrates nor oxygen, while anoxia means only absence of oxygen.
Anaerobiosis step : these aerobic bacteria are stressed, they excrete a quantity Q1 of P in the medium
Aerobiosis step : they incorporate a quantity Q2 of P, and Q2 >> Q1.
P is finally extracted from the plant in the same time than phosphated sludge (which treatment should be aerobic).

Figure 19 illustrates a plant which removes BOD, N and P from MWW.

4.4. Bulking

Bacteria growth can present three forms :
- flocculating growth, which should be obtained for activated sludge process ;
- dispersed growth which occurs when the activated sludge process is started (for the first weeks, before flocculation) ;
- filamentous growth, also called bulking, which occurs with dysfunctions such as presence of toxic chemical in MWW, insufficient aeration of the tank…; this type of growth cause a sharp drop in the quality of treated water due to massive entrainment of SS outside the settling tank.

Figures 20 to 25 illustrates the main filamentous bacteria responsible for bulking in MWW treatment. Their identification can help to know the origin of the dysfunction.

5. Aerobic attached growth : trickling filters, biofilters, biodisks

Among these three technologies, only biofiltration **(figure 26)** is an efficient process : it allows to remove both SS and BOD from MWW ; sometime, nitrification and denitrification can occur.

Biofiltration is an expensive, discontinuous process ; it is well adapted to large variation of load, and can be implanted in specific geographic area like mountains…
The medium can be either sand, or granular activated carbon, or polystyrene balls…on which biofilm takes place.

Trickling filters technology (**figure 27**) is quiet rare : biofilm is fixed on large pozzolan or plastic stones and SS are not removed from MWW ; it is often used as a pretreatment before activated sludge, for very concentrated industrial waste water (high BOD).

Biodiscs technology **(figure 28**) is also rare : the biomass is attached to discs that turn around a horizontal axis and are partially bathed in raw water ; rotation brings the biomass alternately in contact with the water to be treated and the oxygen in the air. Discs are spaced 2 or 3 cm from one another and tun at 1 or 2 rpm ; they are 2 or 3 m in diameter and made of polystyrene ; they must be cover to protect them against harsh weather. This process consumes little electrical energy.
A downstream clarifier retains the excess sludge.

6. Extensive processes : lagooning

Natural biological purification of pollutants can occur in water : in this way, many different metabolisms are involved ; in fact, the food chain is the same as the activated sludge one but the source of oxygen is photosynthesis and not artificial aeration.

The Winograsky column illustrates all the nutritional types which can occur in a layer of water :
figure 29.
All these natural biological reactions are exploited in lagooning ; **figure30** illustrates such a two-step process : in a first microphytes lagoon, SS settle, some fermentations occur ; on the surface, algae generates oxygen which allow aerobic metabolism like BOD removal and nitrification ; the second step consists in assimilation of nitrates and phosphates by macrophytes.
This technology needs a lot of space (10 m^2 / habitant).

7. Anaerobic bacterial growth for industrial waste water purification and sludge stabilization

In anaerobic conditions, organic matter is reduced to methane CH_4. This natural metabolism occurs in many different places, like in deep ocean, in the ruminants rumen…
Because of the very slow growth of methane-producing bacteria and the cost of the industrial installation, it is applied for very high BOD load, i.e. for industrial waste water (agro-food) or for sludge stabilization in large plants.

7.1. Biochemistry of the process

Figure 31 summarizes the different biochemical ways leading to methane : only some of these ways are used in a specific place (note that their should be competitions, for example for H_2 between acetate- producing bacteria and some methane - producing bacteria).
In a digester, the following ways take place :
- acetogenesis OHPA (and not the homoacetic acetogenesis)
- both methanogenesis.

Methane – producing bacteria are archaebacteria, strictly anaerobic ; their generation time is very long (15 to 30 days).

7.2. Industrial waste water purification : suspended and attached growth

Figure 32 illustrates a mixed digester (suspended growth) and settling tank : Analift (Degrémont) ; the degasification device is required to remove the occluded gas, which hinders settling, from the floc.
It is suitable for concentrated effluents (distilleries…). The COD load varies from 3 to 15 kg / m^3 . d.

Figure 33a illustrates an attached growth on a support medium : the biofilm grows on a fixed medium, through which the water passes in upflow : Anafiz (Degrémont) ; it is suitable for relatively diluted effluents such as dairies, sweet factories…The COD load varies from 8 to 15 kg / m^3 . d.

Figure 33b illustrates an attached growth on fluidized bed : Anaflux system, Degrémont. The advantages of the process are :
- no risk of clogging of the support
- rapid start-up
-compact unit
- accommodation of considerable flow variation.
It is suitable for effluents from breweries, canning factories… ; the COD load varies from 30 to 60 kg / m^3 . d.

7.3. Sludge stabilization

Biological sludge stabilization (reduction of the pathogenic organisms and of organic matter concentration) can be performed in three ways ;
- aerobic process in a structure equivalent to the activated sludge : **figure 34**
- anaerobic process, number of steps depending of the load : **figures 35 and 36**
- composting : **figure 37**

Conclusion : the **figure 38** summarizes all the biological processes applied to waste water treatment.

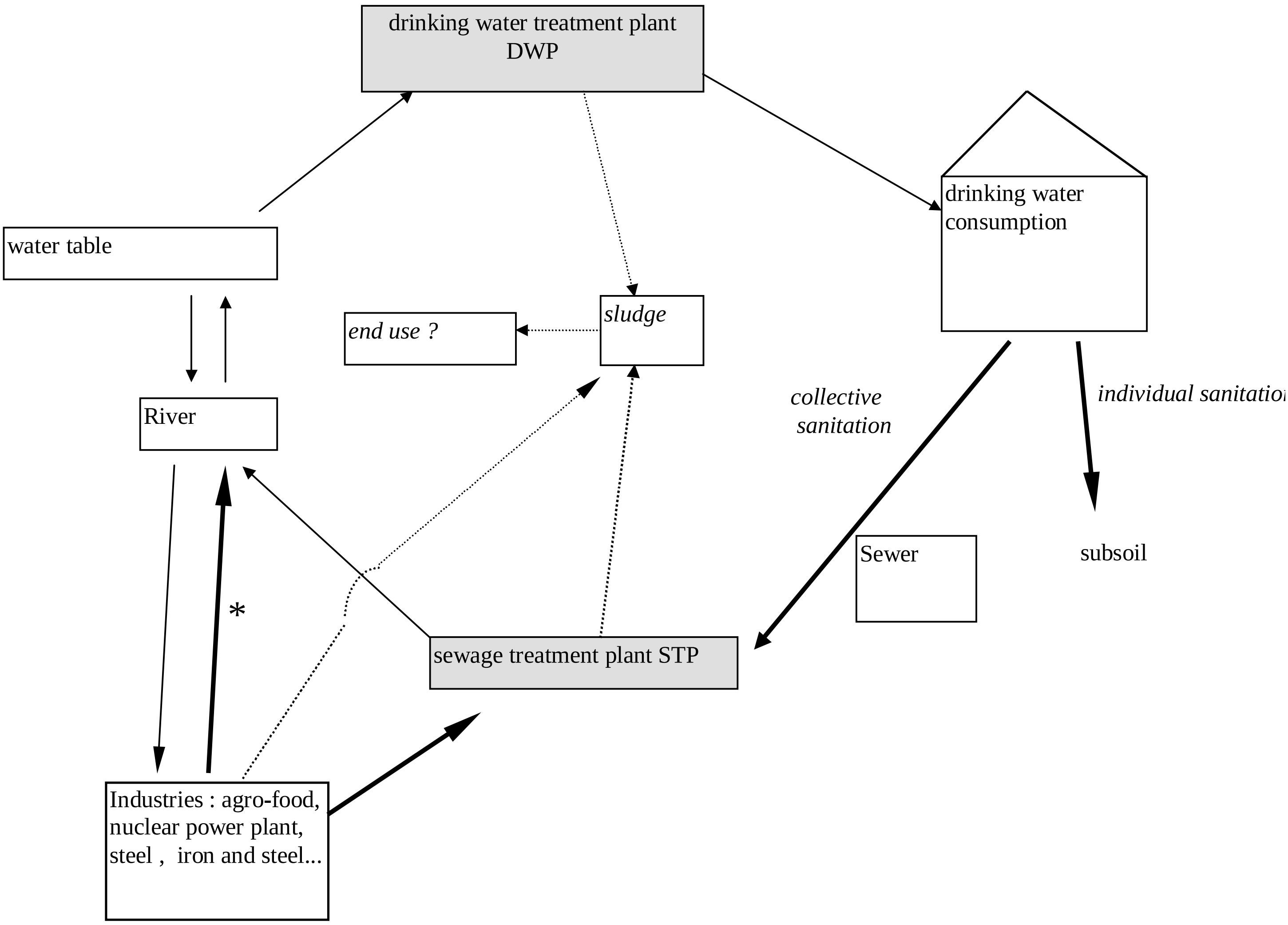


Figure 1 : Water cycle in town

Caption :

raw water for DWP or industries

municipal and industrial waste water ;* the necessity to treat industrial waste water depends on its quality and the regulation

sludge

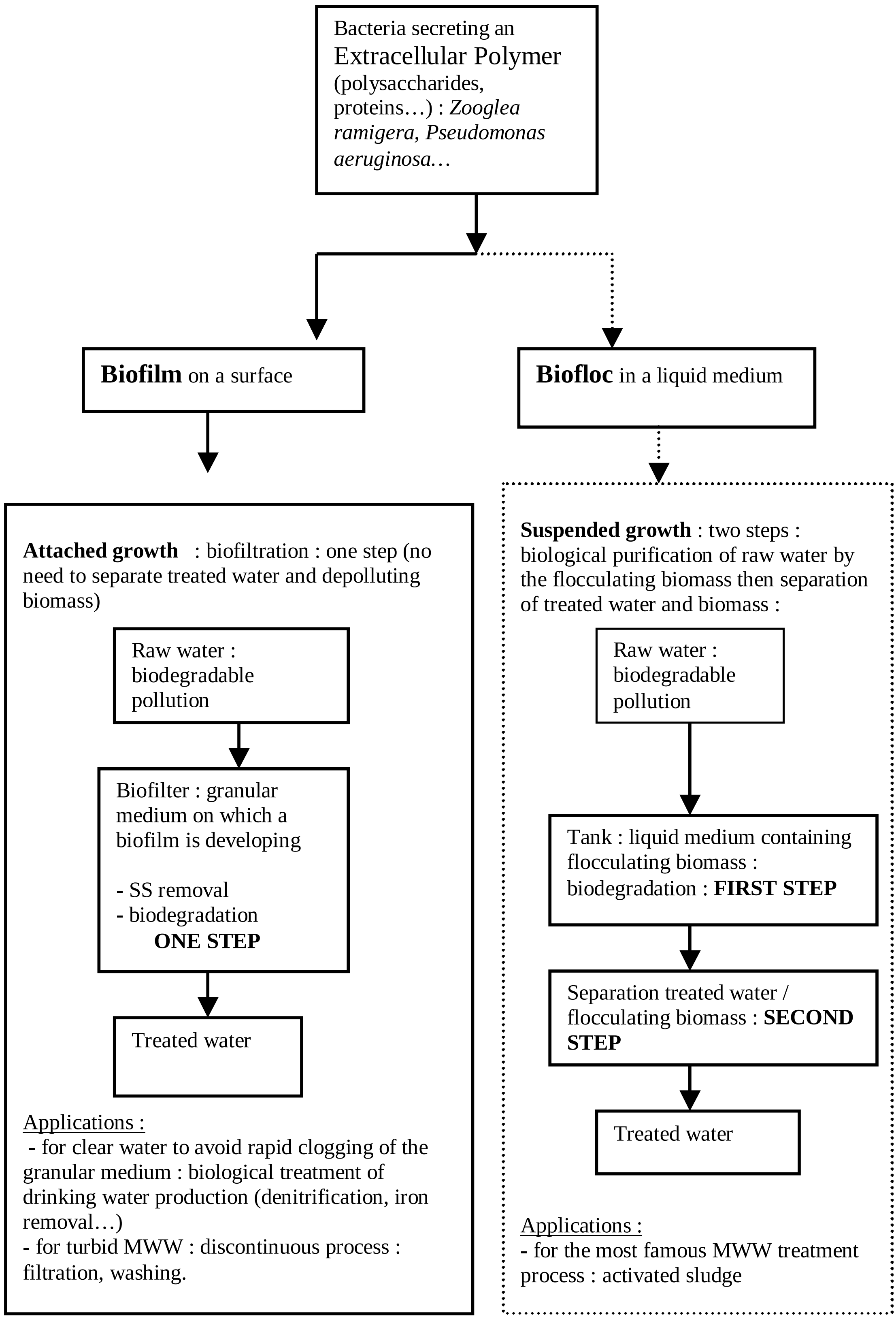


Figure 2 : Attached and suspended growth in water treatment

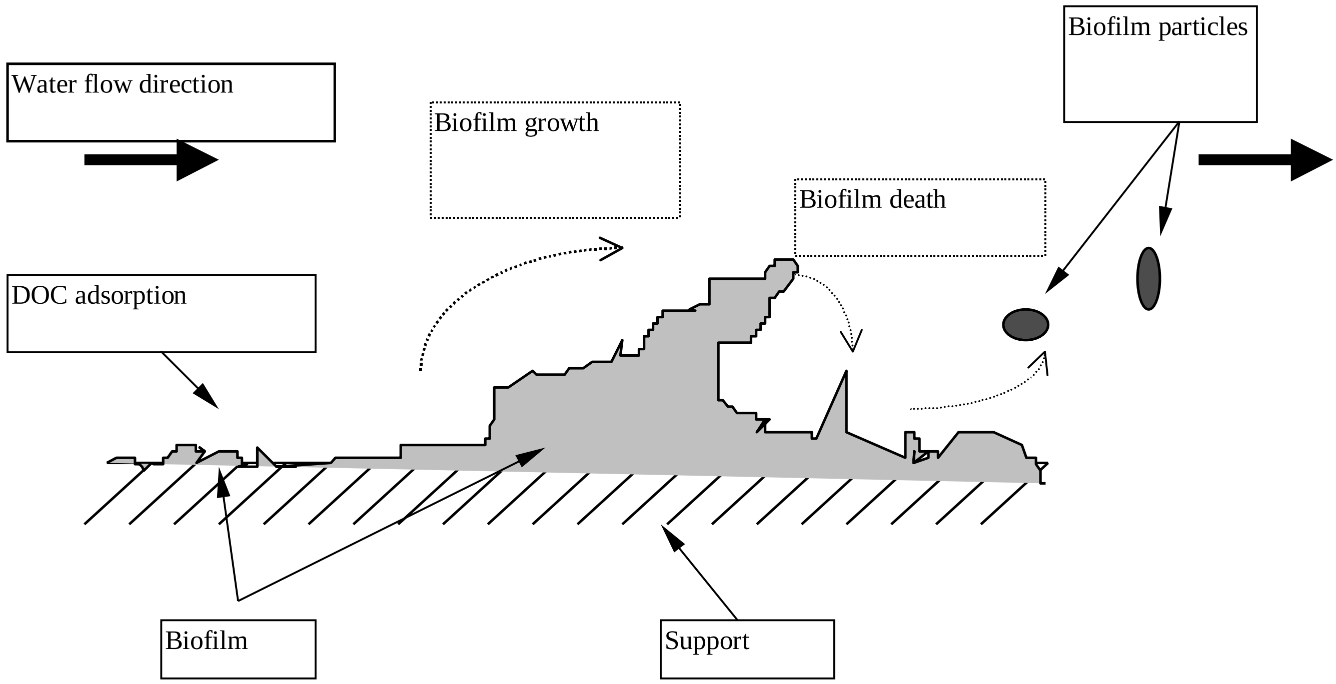


Figure 3 : Sketch of a biofilm (Maul A., Vagost D., Block J.C., 1989)

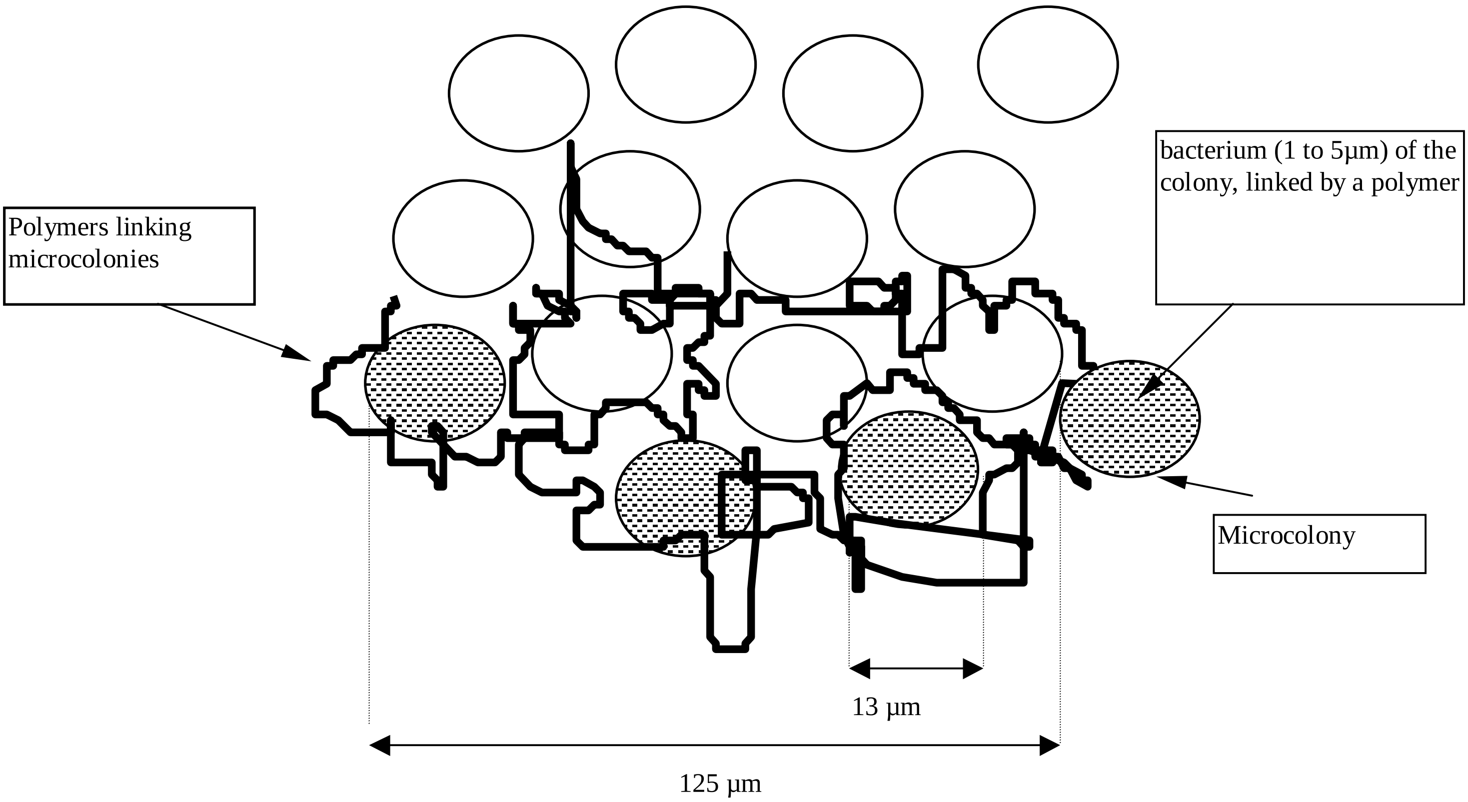


Figure 4 : Model of structure microbial floc in activated sludge

Figures 5 to 14: brief phenetic classification of microfauna in activated sludge
The considered taxonomic hierarchy is :
Kingdom: animal
Sub kingdom
Branch
Class
Sub class
Order
Family
Genus

Sub kingdom	PROTOZOAN							METAZOAN
Branch	Rhizopoda			Flagellata	Ciliata			worms
Class, sub class or order	heliozoan	amoebiens	thecamoebiens	Zoo flagellated	holotrichs	peritrichs	hypotrichs	**Rotifers,** Gastrotrichs ***Nematodes***
Most famous genus in activated sludge microfauna		***Amoeba***	***Thecamoeba***	Pleuromonas Bodo, Monosiga	***Paramecium, Trachelophylum*** Lionotus, Chilodonella	***Vorticella,*** Carchesium, ***Episitylis***, Opercularia	Euplots, Aspidisca	

In thick letters are the organisms studied below ; for each one, are detailed :

- a sketch and the average size of the organism
- its phenetic classification
- the predation, the habitat of the microorganism and the relation with the process management

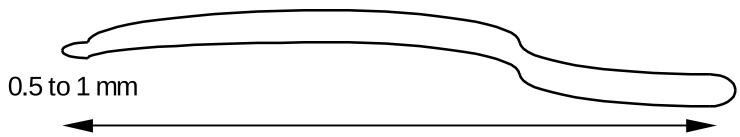


Figure 5 : Sketch of an organism belonging to the class of nematodes (branch of worms)

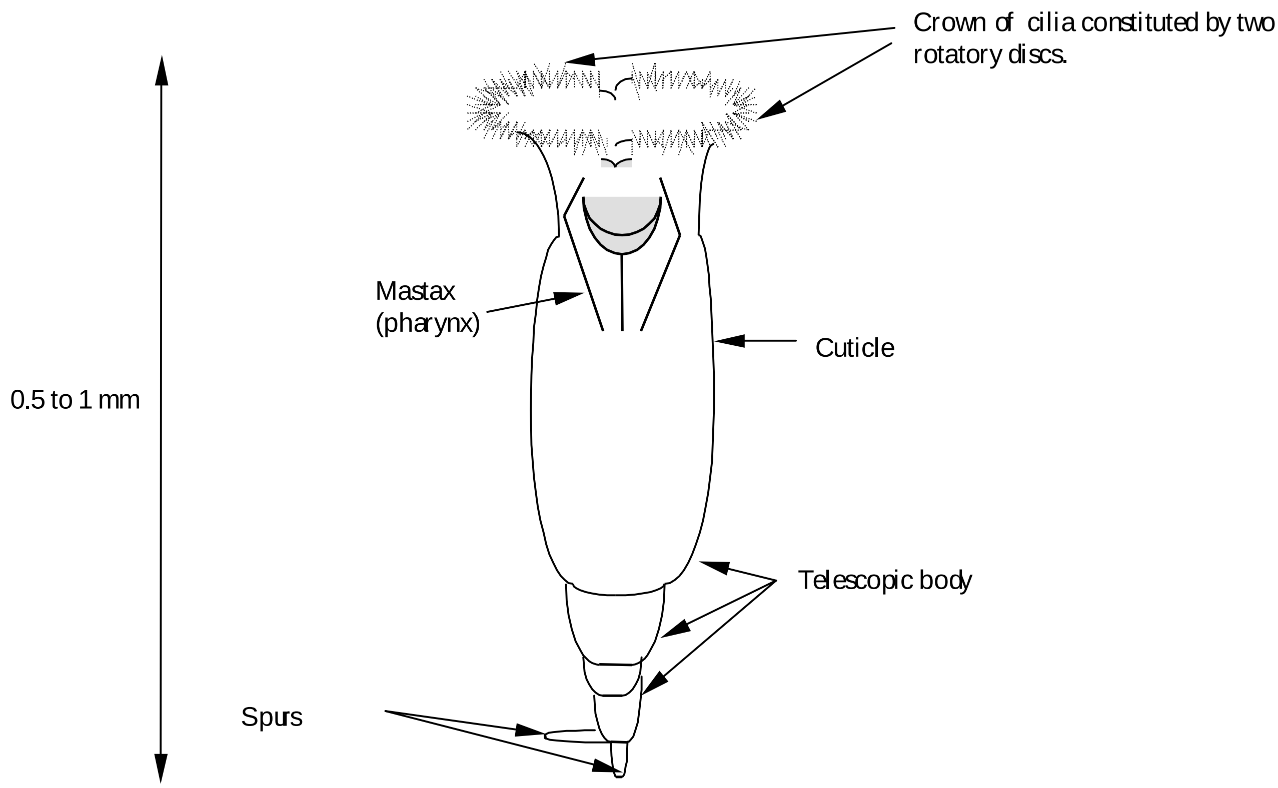


Figure 6 : Sketch of a metazoan organism

* Sub kingdom of metazoa, branch of worms, class of rotifers, fifteen known genus

* bacterio- or protozoophagous ; planktonic or fixed species ; low load and high sludge age : satisfying treatment efficiency and nitrification

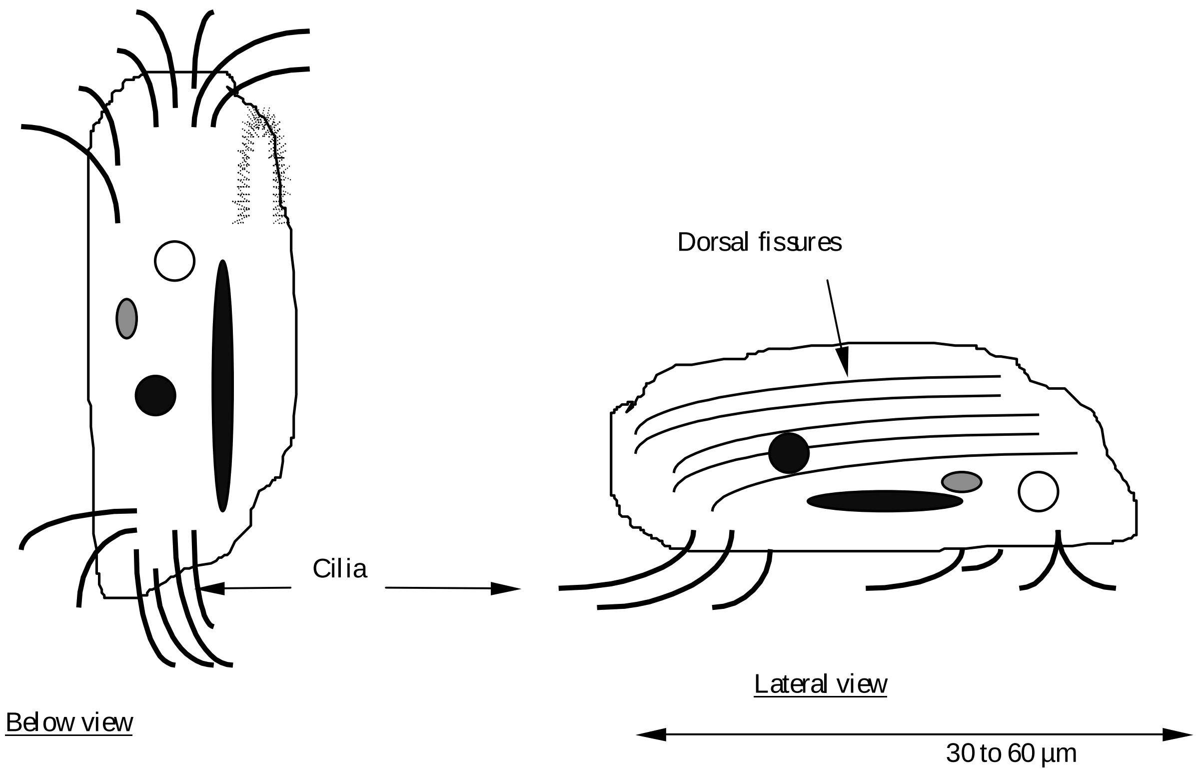


Figure 7 : Sketch of a protozoan organism

* class ciliate, sub-class of hypotrichs (seven known genus, main are Euplotes and Aspidisca)

* bacteriophagous ; adapted to the surface of the flocs, mobile ; low load and high sludge

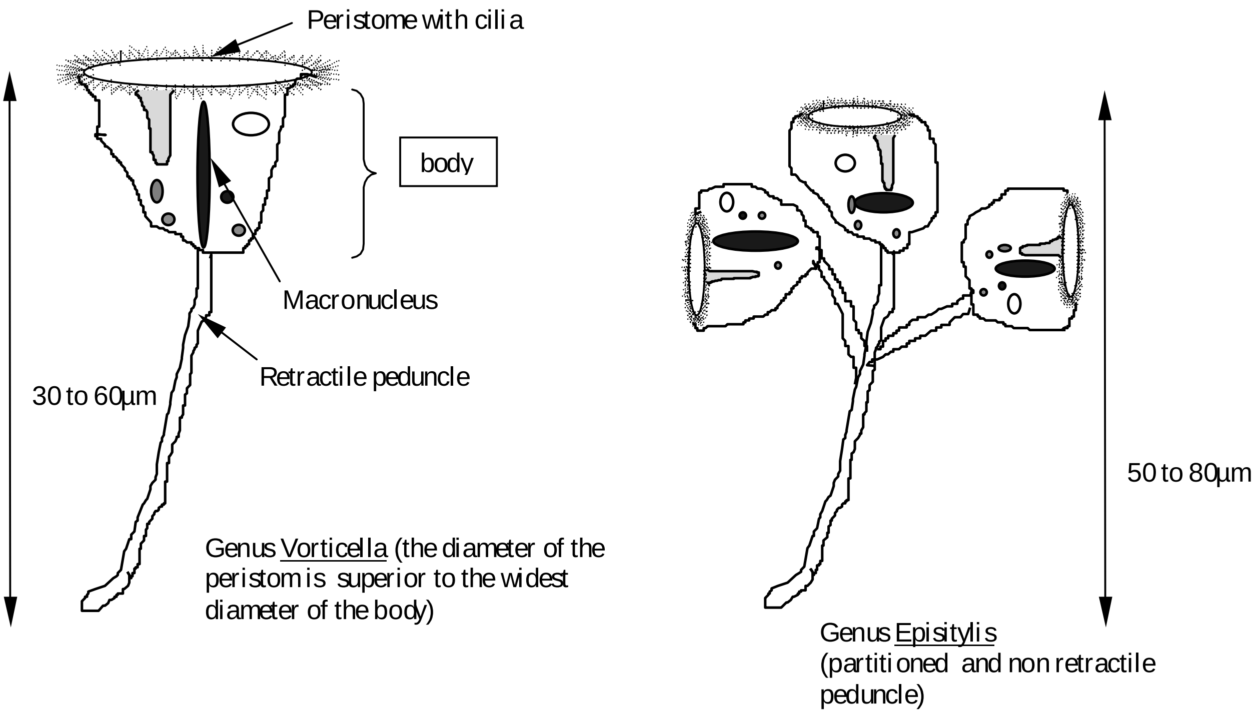


Figure 8 : Sketch of a protozoan organism
* class of ciliate, sub-class of peritichs (six known genus)
* bacteriophagous (free bacteria), fixed at the surface of the floc, low load, well a aerated medium

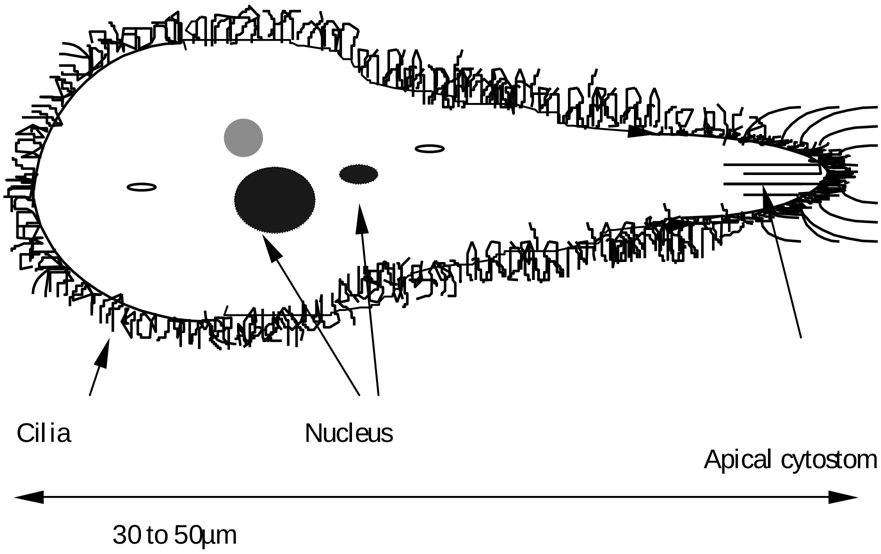


Figure 9 : Sketch of a protozoan organism permanent in microfauna of activated sludge

* class of ciliate, sub-class of holotrichs, genus Trachelophylum
* adapted to the surface of the floc but no fixed and free swiming, bacterio- and protozoophagous ; high concentration of oxygen, moderate or high load .

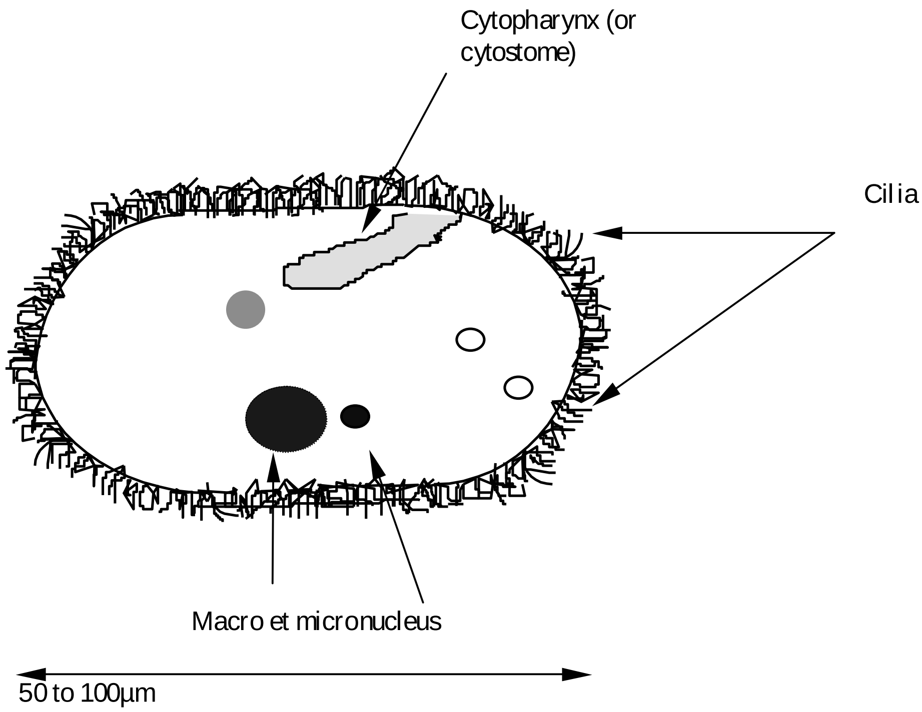


Figure 10 : Sketch of a protozoan organism : Paramecium

* class of ciliate, sub-class of holotrichs
* bacteriophagous ; swimer ; need a lot of oxygen, low load.

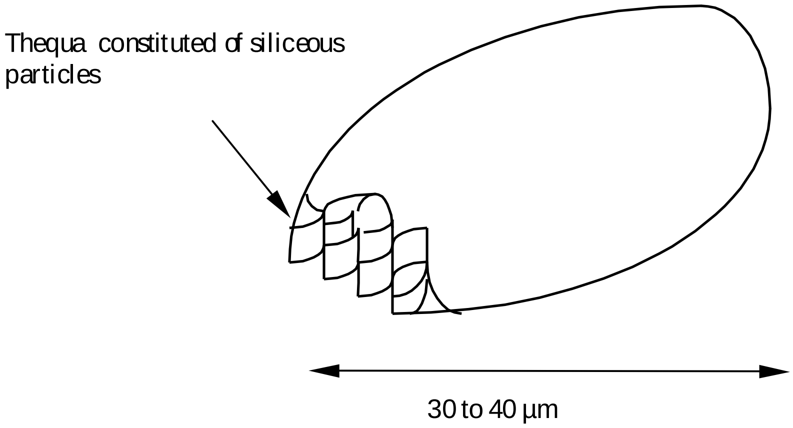


Figure 11 : Sketch of a protozoan organism
* branch of Rhizopoda, class of Thaecamoeba, genus Euglypha
* living on flocs, no fixed, no swimer, bacteriophagous (some consume filamentous bacteria) ; stable sludge, low charge.

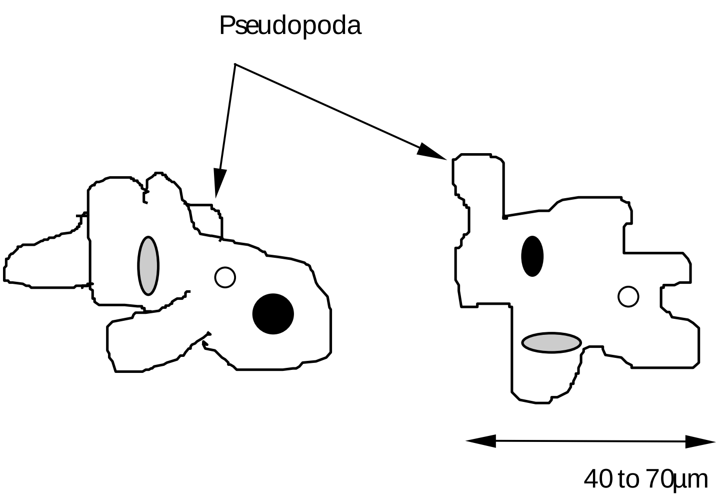


Figure 12 : Sketch of a protozoan organism : Amoeba
* branch of Rhizopoda, class of amoeba
* bacterio- ou protozoophagous ; live on the surface of flocs ; few indication about its relation with the quality of the treatment

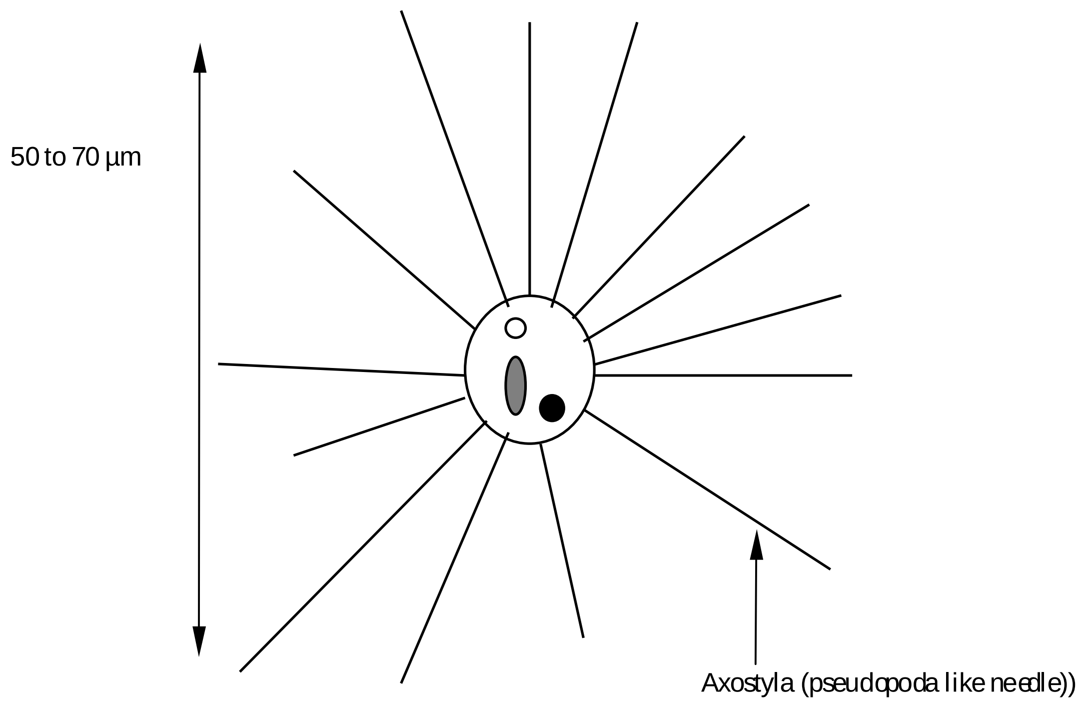


Figure 13 : Sketch of a protozoan organism : Heliozoa
* branch of Rhizopoda
* bacteriophagous ; rare in sludge, planktonic ; low load and high sludge.

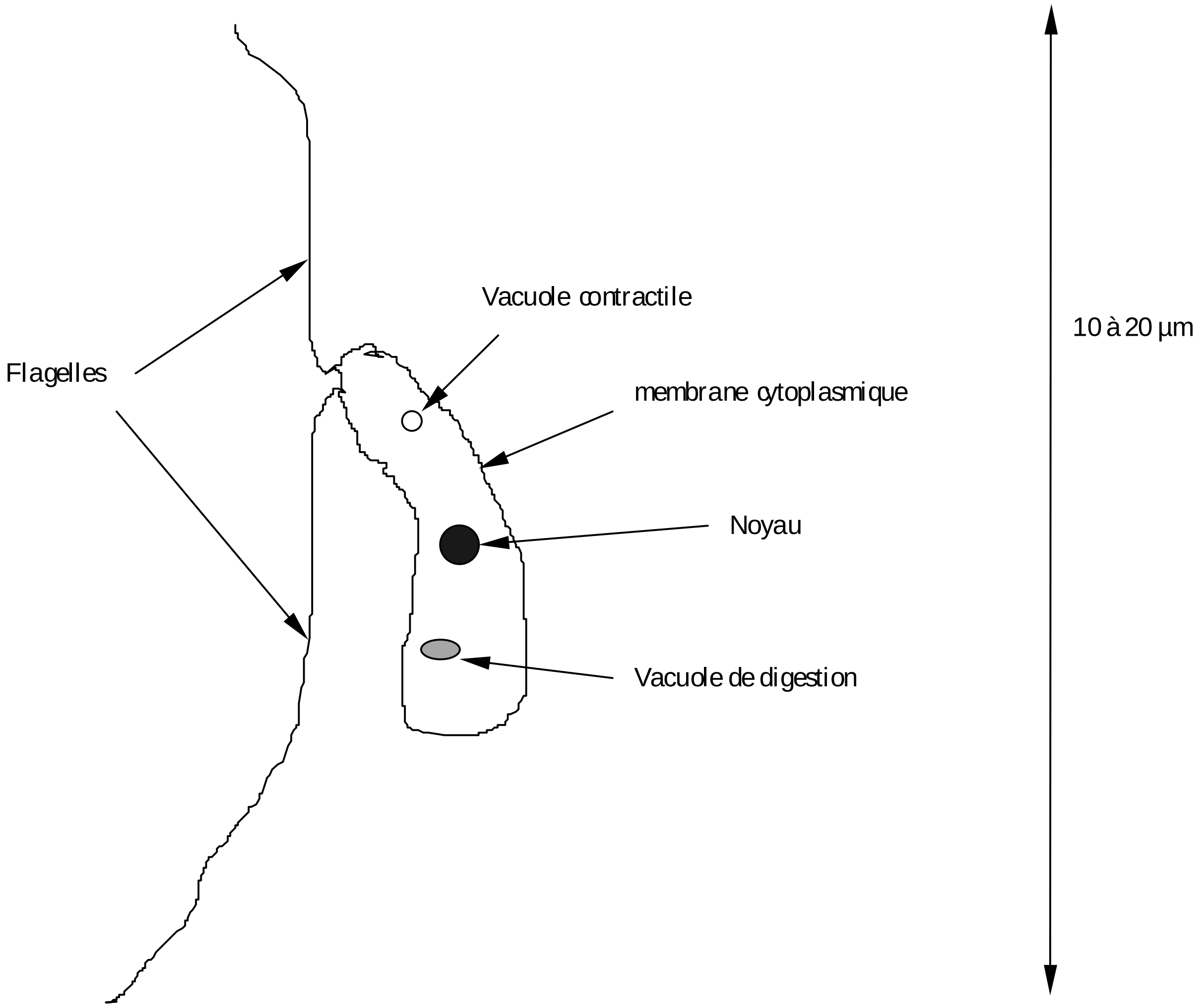


Figure 14 : Sketch of a protozoan organism:
* branch of flagella, class of zooflagellates
* swimmer, consumes organic matters and bacteria ; very young sludge, or adapted to IWW containing phenol

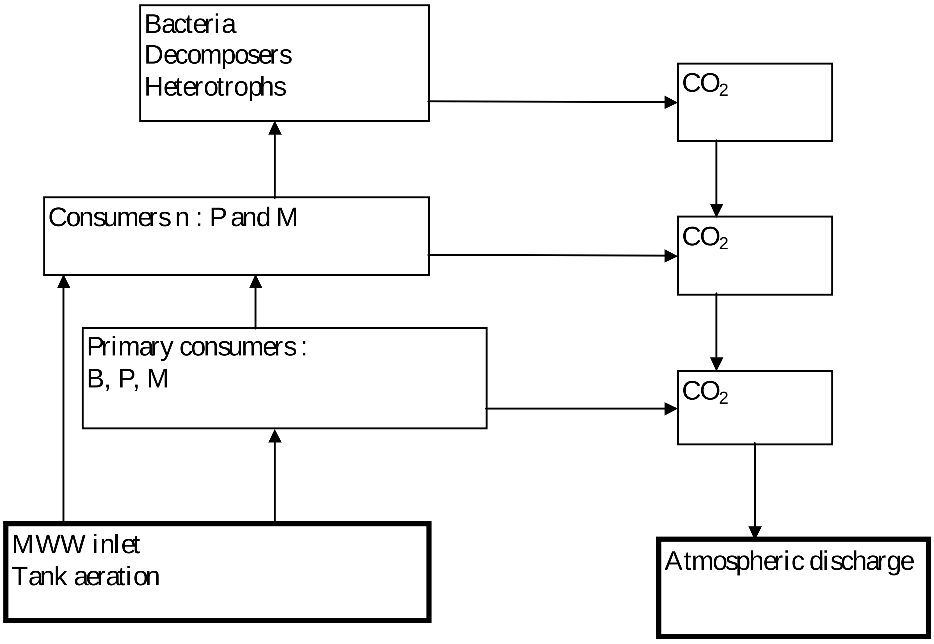
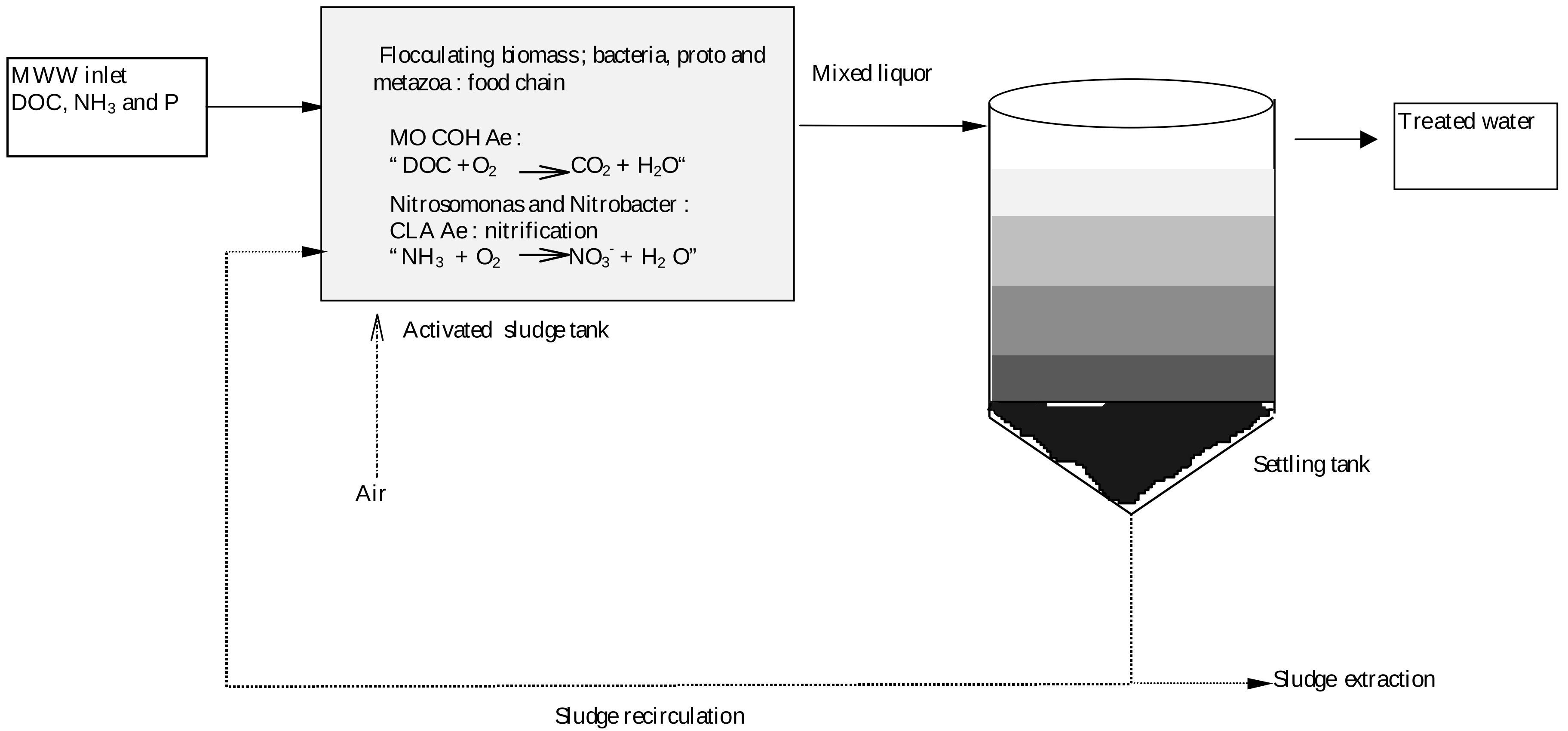


Figure 15 : Food chain in an activated sludge aerated tank

Caption:
B : bacterium
P : protozoan
M : Metazoan



<u>Figure 16 : Sketch of a structure of MWW treatment with activated sludge process (biology)</u>

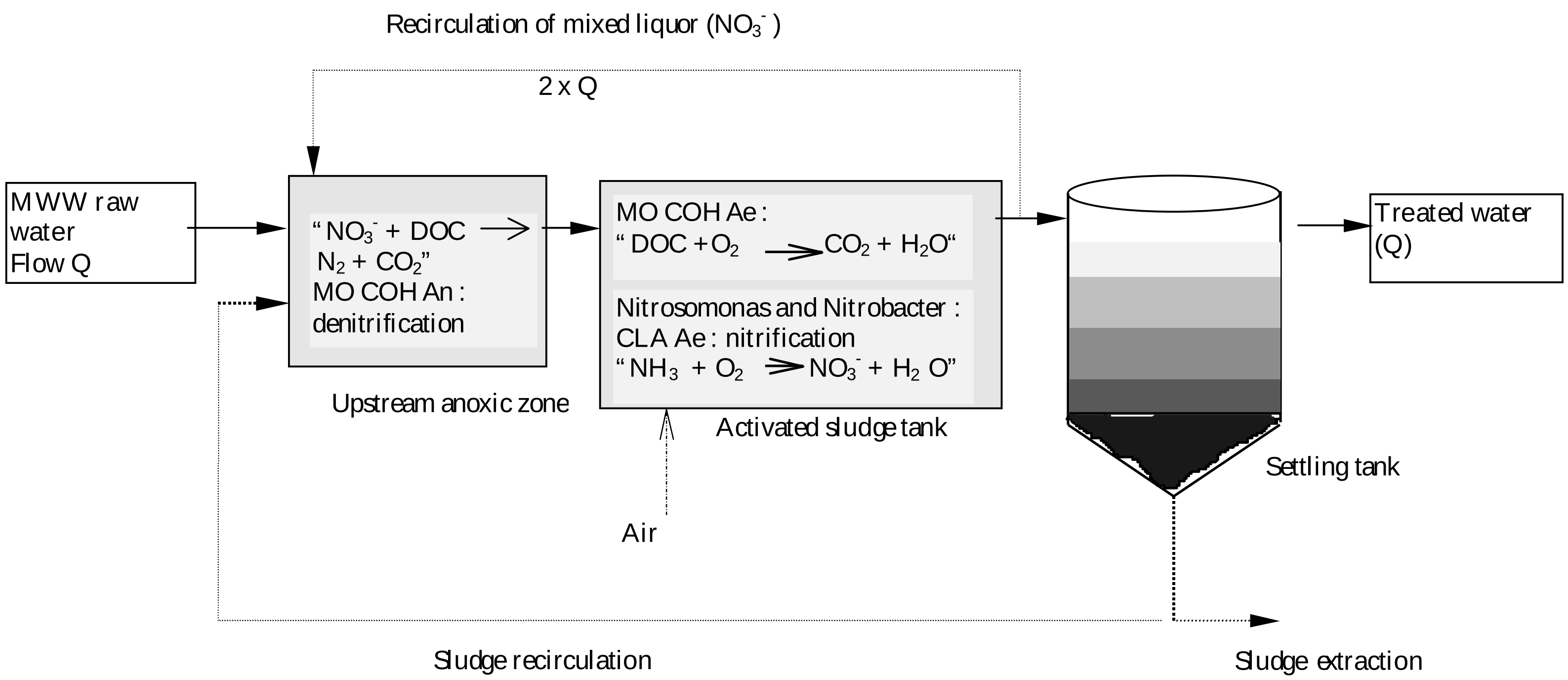


Figure 17 : Sketch of a structure of denitrification in an upstream anoxic zone

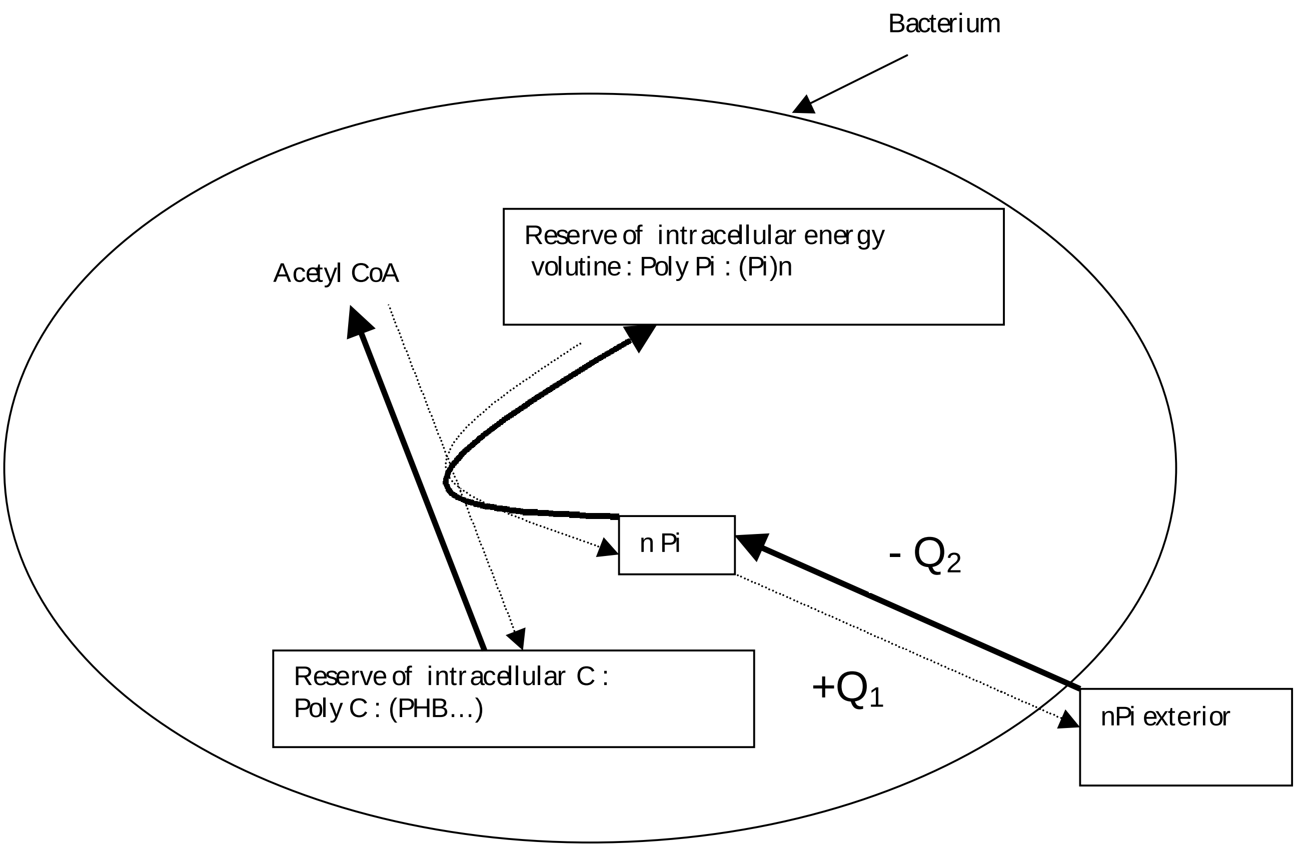


Figure 18 : Simplified sketch of phenomena involved in biological phosphate removal in water (Comeau et al., 1986)

Caption :

metabolism in anaerobiosis

metabolism in aerobiosis

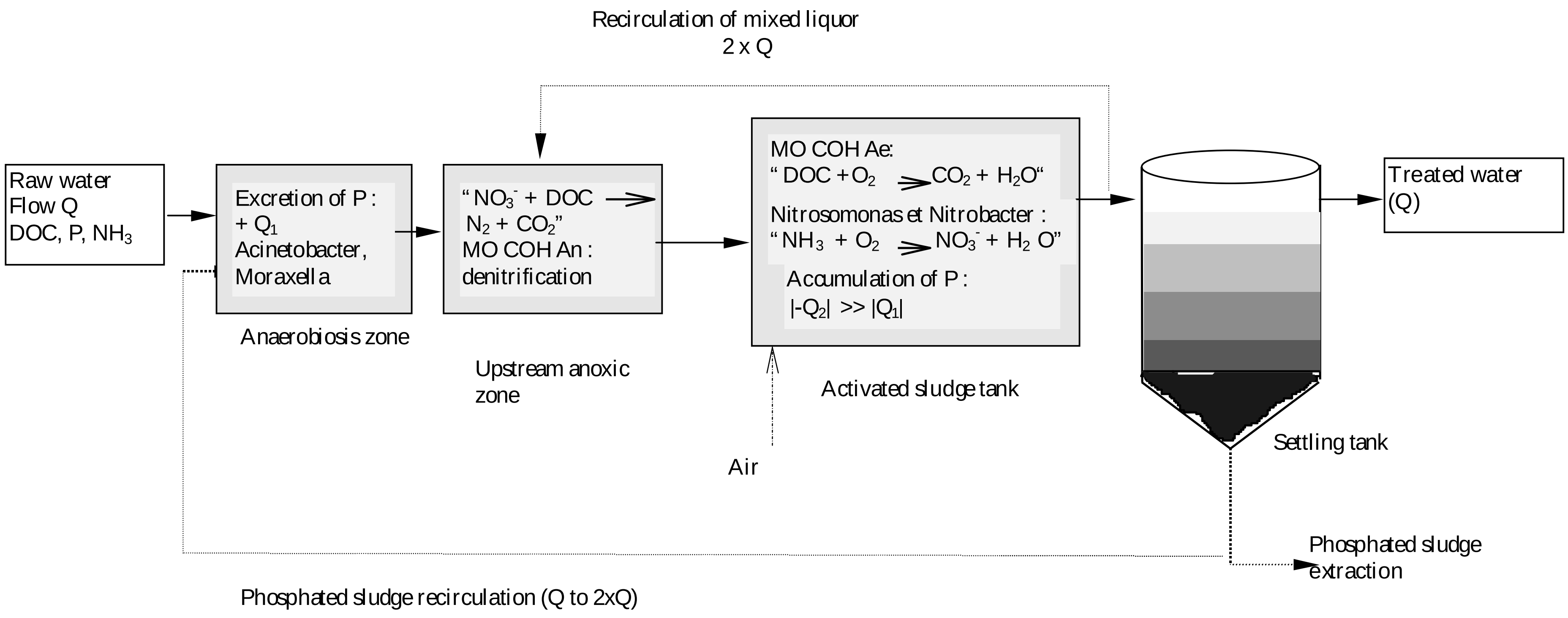


Figure 19 : Sketch of a structure of biological removal of DOC, N and P in MWW

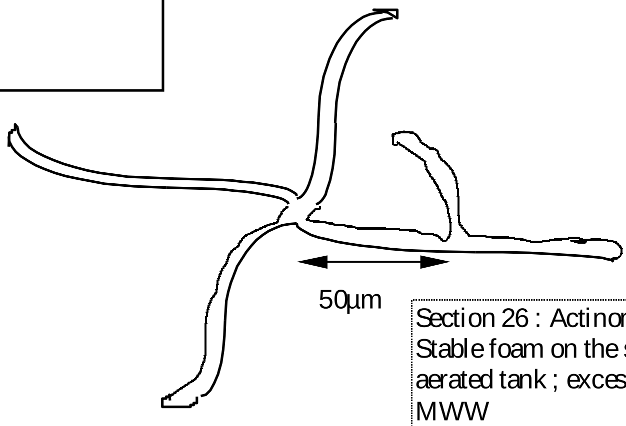
Figures 20 to 25 : Main filamentous bacteria responsible for bulking in activated sludge processes

* sketch

* morphology, Gram stain, Neisser stain , sulphur granule determination

* classification (Bergey's Manual of Systematic Bacteriology, J.G. Holt et N.R. Krieg, 1984-1989) and relationship with water treatment

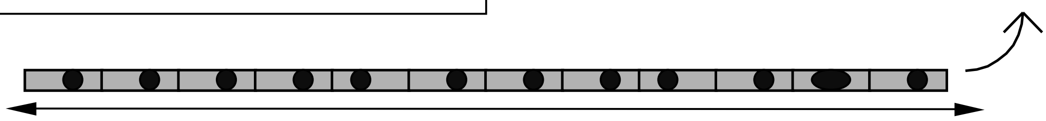
Nocardia sp. : short and branched filament , non partitioned ,G+, N+, S-



Section 26 : Actinomycetal
Stable foam on the surface of the aerated tank ; excess of grease in MWW

Figure 20

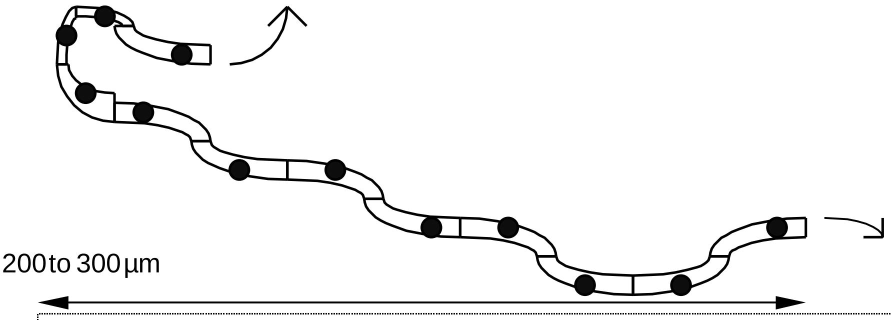
Thiothrix sp : straight or not much curved filament , non branched, without sheath, partitioned, sulphur granule (●), G-, N-, S+



Section 23 : gliding bacterium, non photosynthetic and without fructification ; order Beggiatoales
Septic MWW concentrated in sulphured reduced compounds

Figure 21

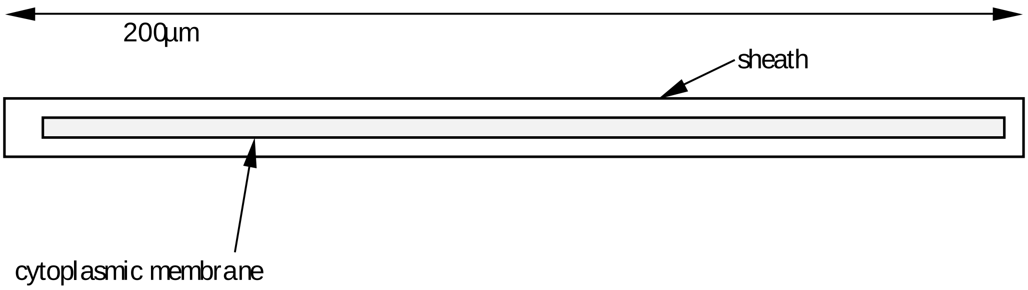
Beggiatoa sp. : flexible filament , mobile, partitioned, sulphur granule,
G-, N-, S+



Section 23 : gliding bacterium, non photosynthetic and without fructification ; order Beggiatoales
Observed in insufficiently aerated attached growth

Figure 22

Haliscomenobacter : Thin and straight filament,
non partitioned , sheath, G-, N-, S-



Section 22 : sheathed bacteria
Insufficiently aerated sludge

Figure 23

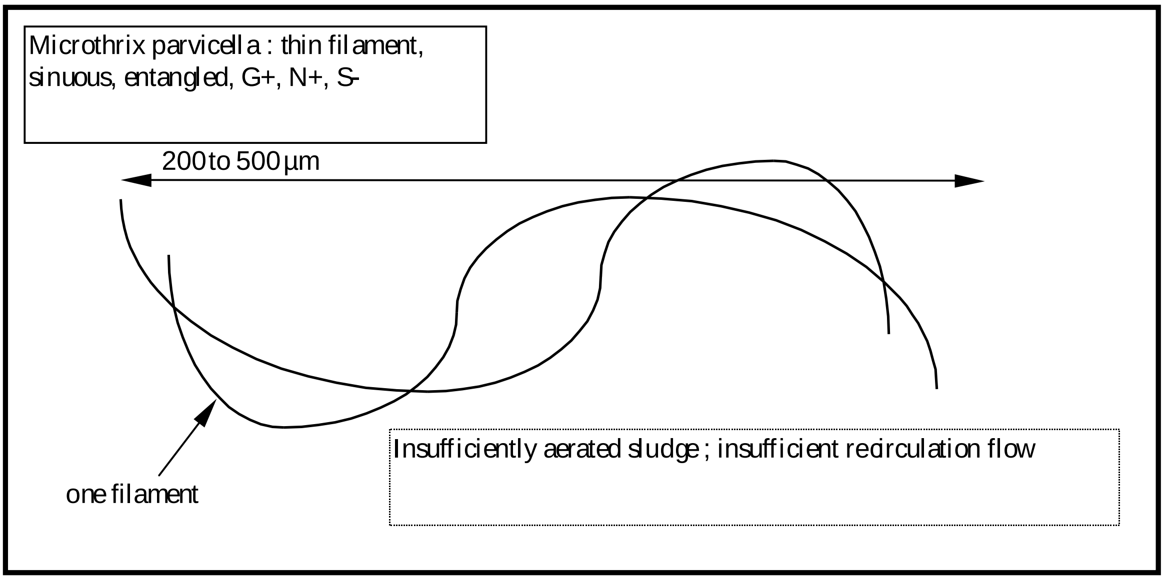


Figure 24

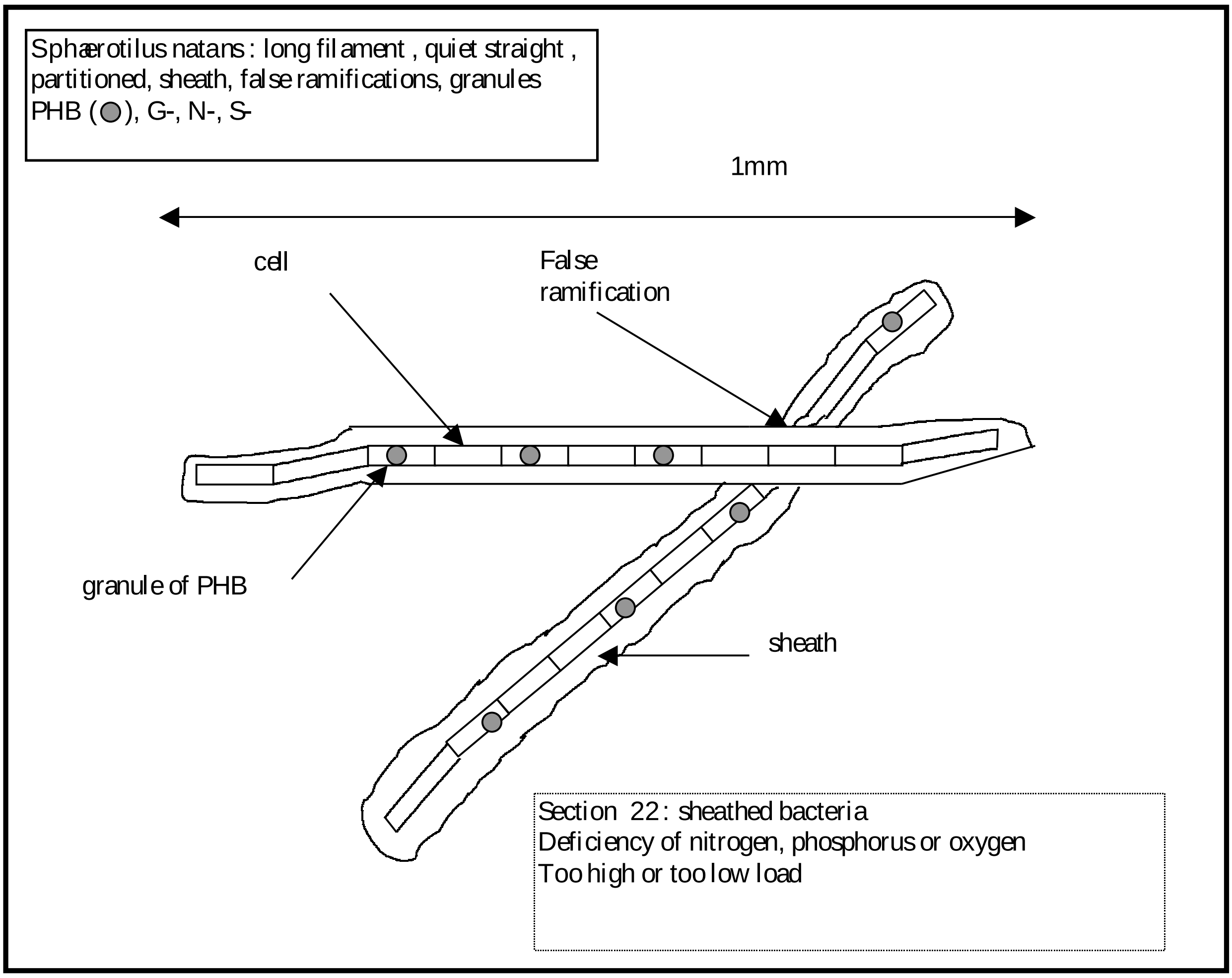


Figure 25

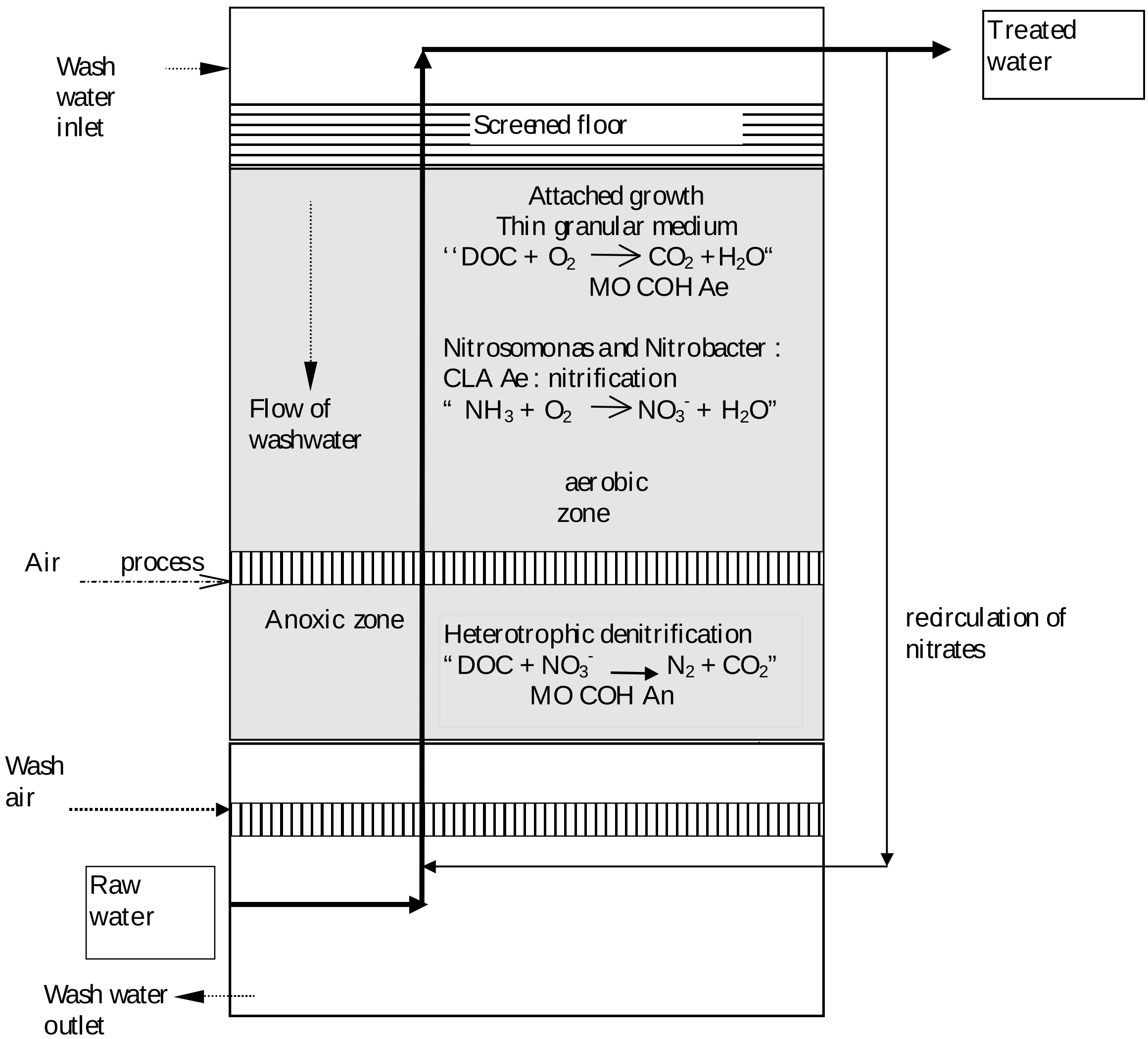


Figure 26 : Sketch of a structure of MWW treatment by biofiltration : biological removal of DOC and N (process Biostyr, OTV)

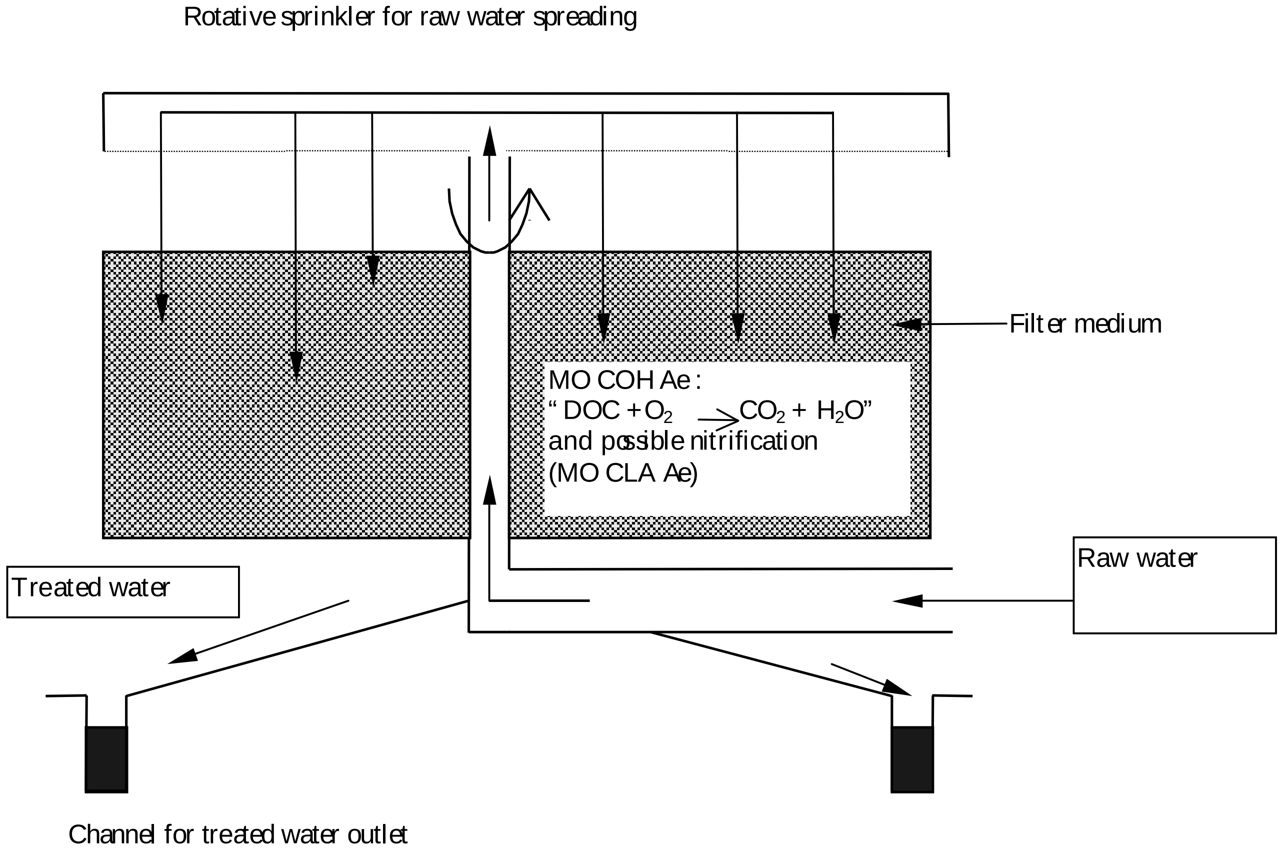


Figure 27 : Sketch of a trickling filter

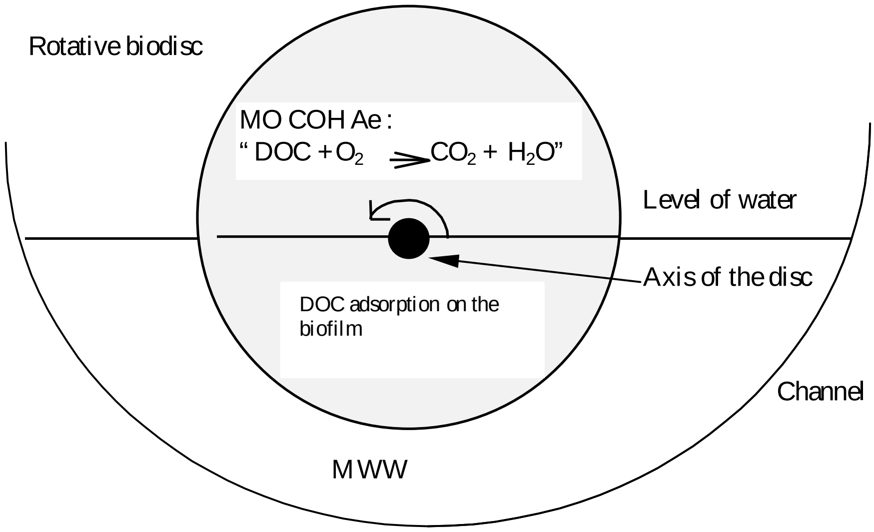


Figure 28 : Sketch of a biodisc ; water circulates in a plane perpendicular to the plane of this sketch , and parallel to the direction of the axis

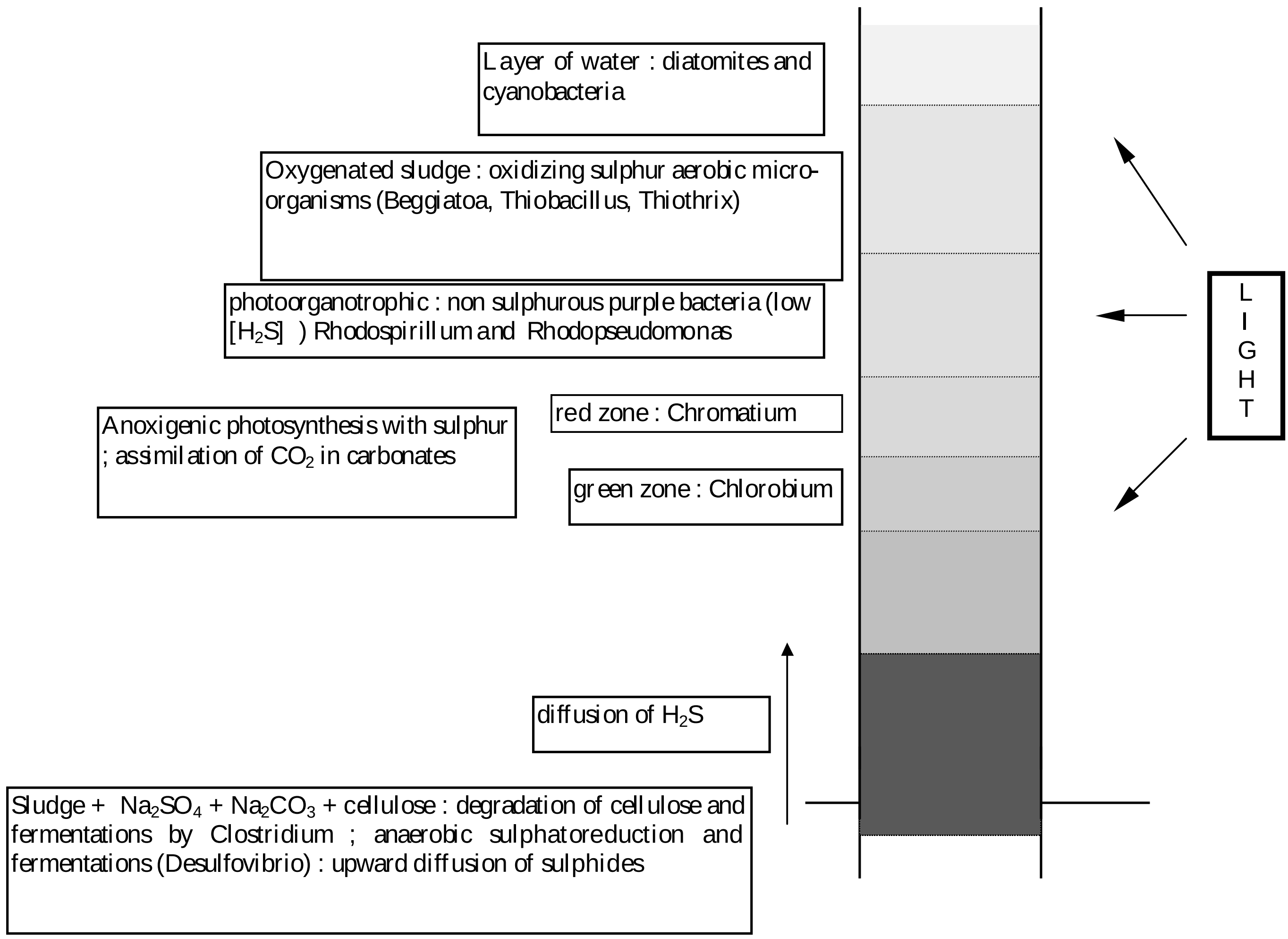


Figure 29 : The Winogradsky column experiment

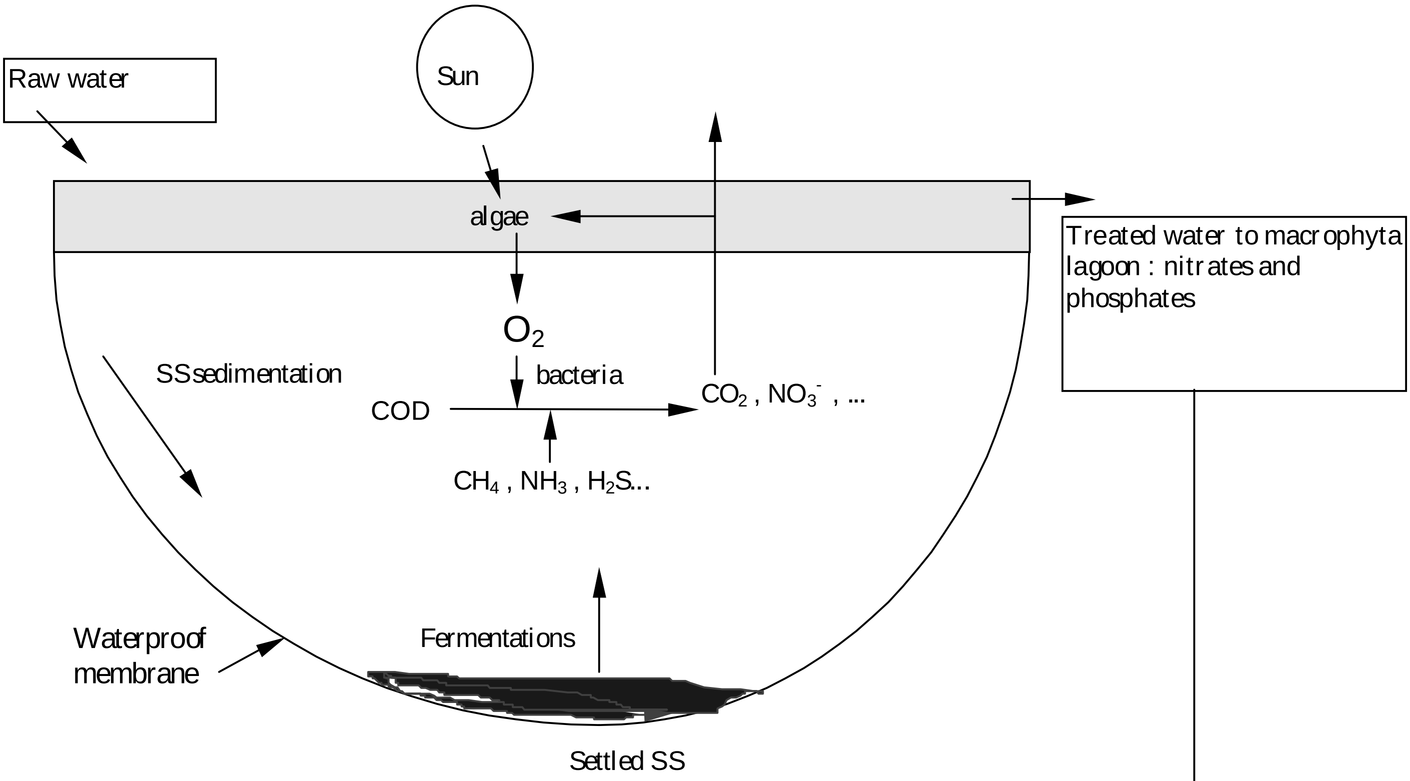


Figure 30a : Sketch of a microphytes lagooning

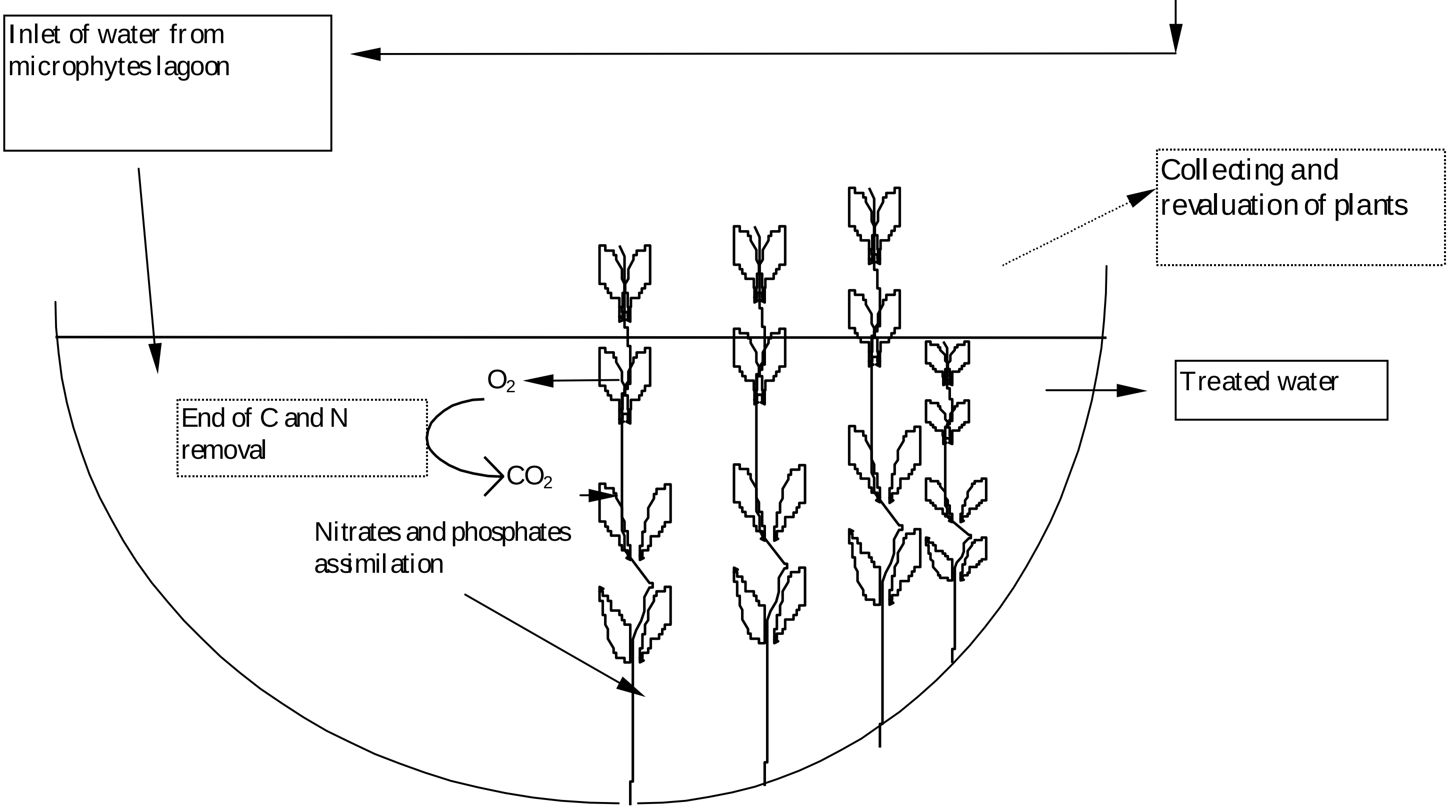


Figure 30b : Sketch of a macrophytes lagooning

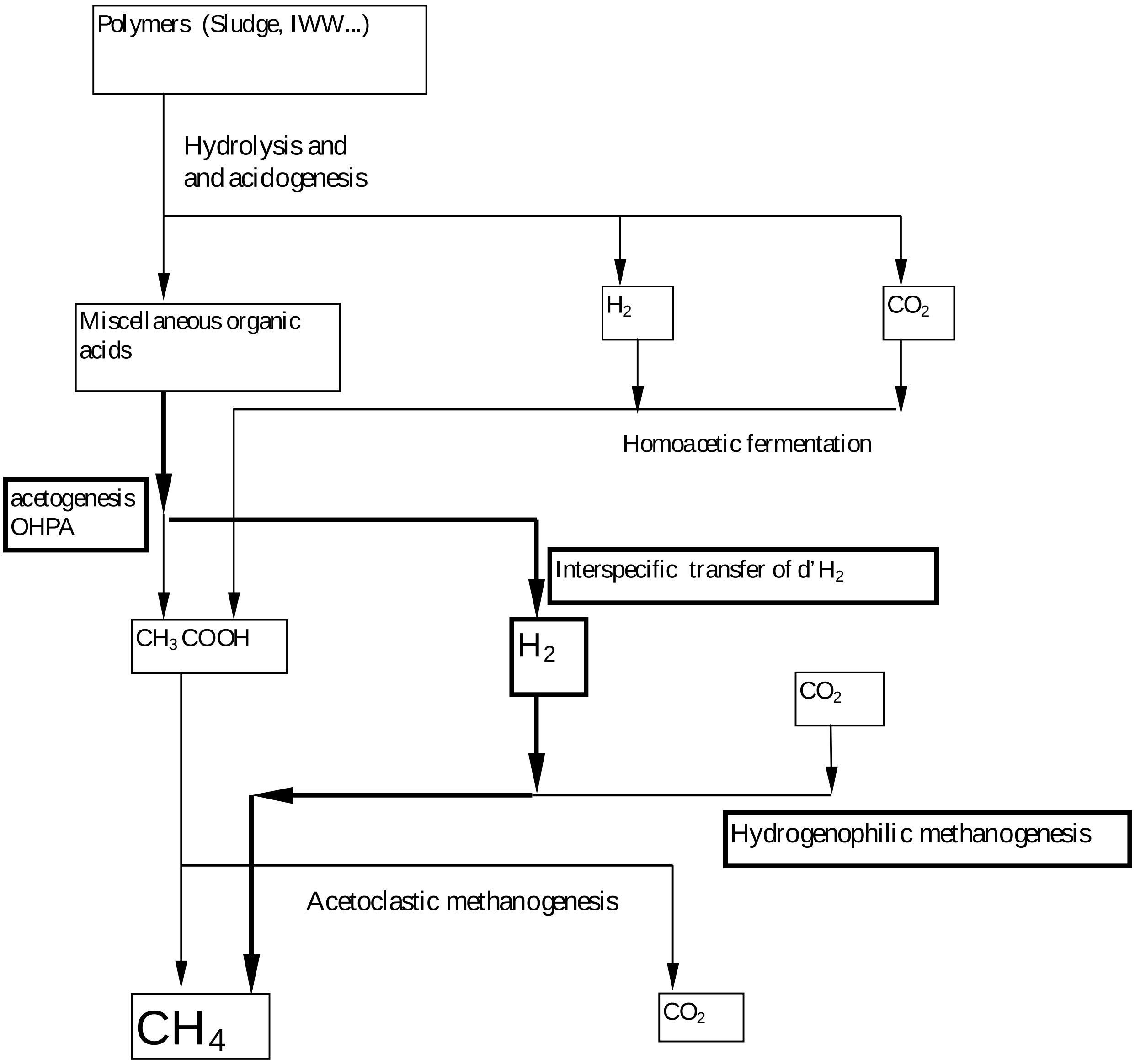


Figure 31 : The different way of anaerobic degradation of organic mater

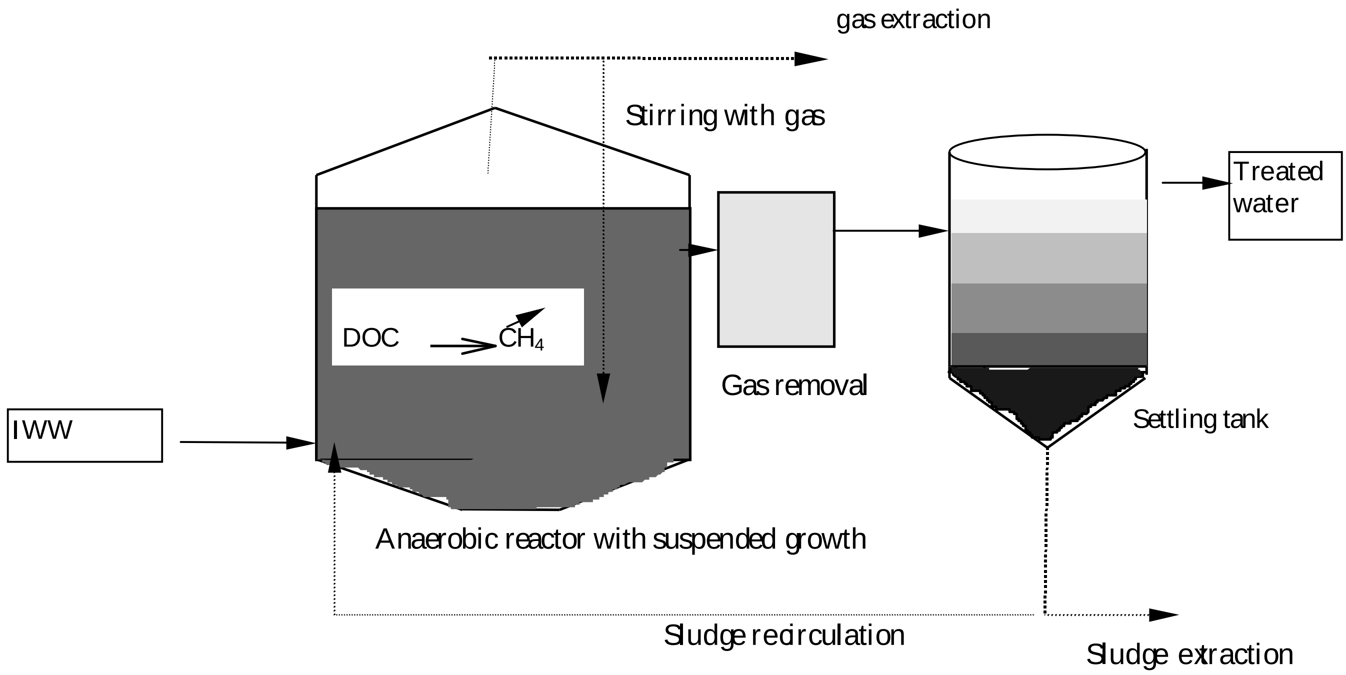


Figure 32 : Sketch of a structure of IWW treatment, with anaerobic digestion suspended growth

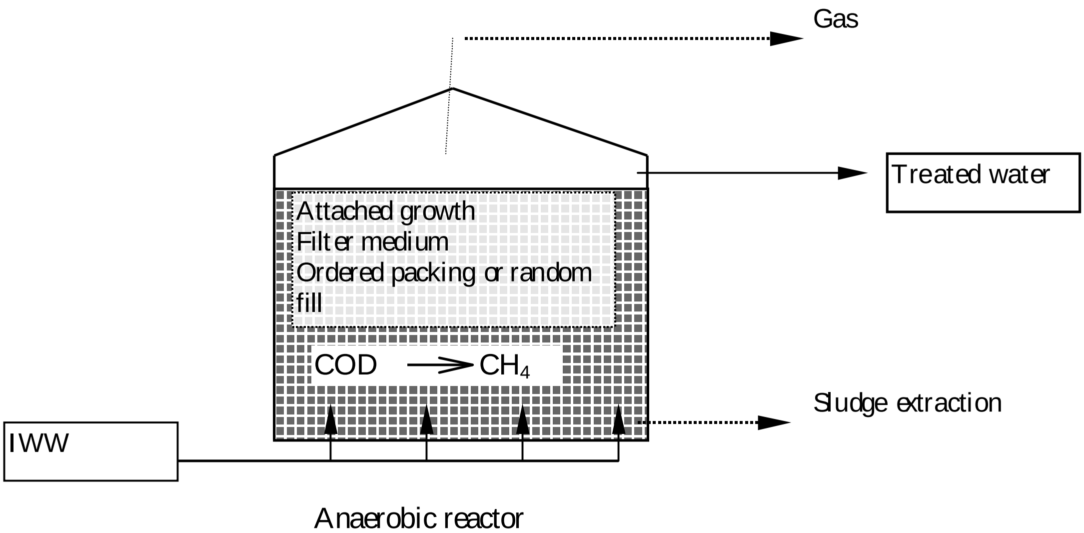


Figure 33a : Sketch of a structure of IWW treatment, with anaerobic digestion attached growth

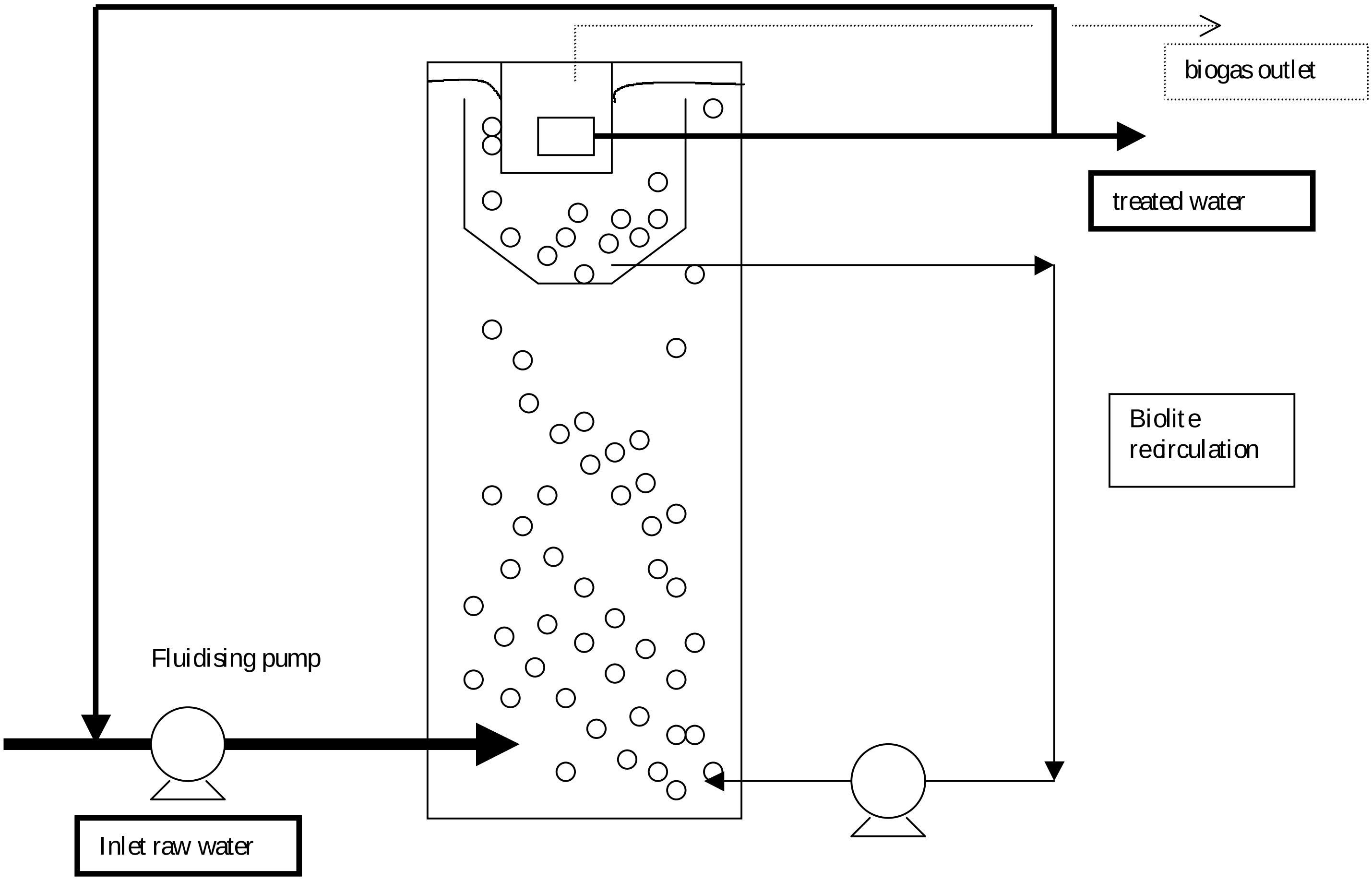


Figure 33b : The Anaflux System (Degrémont, France)

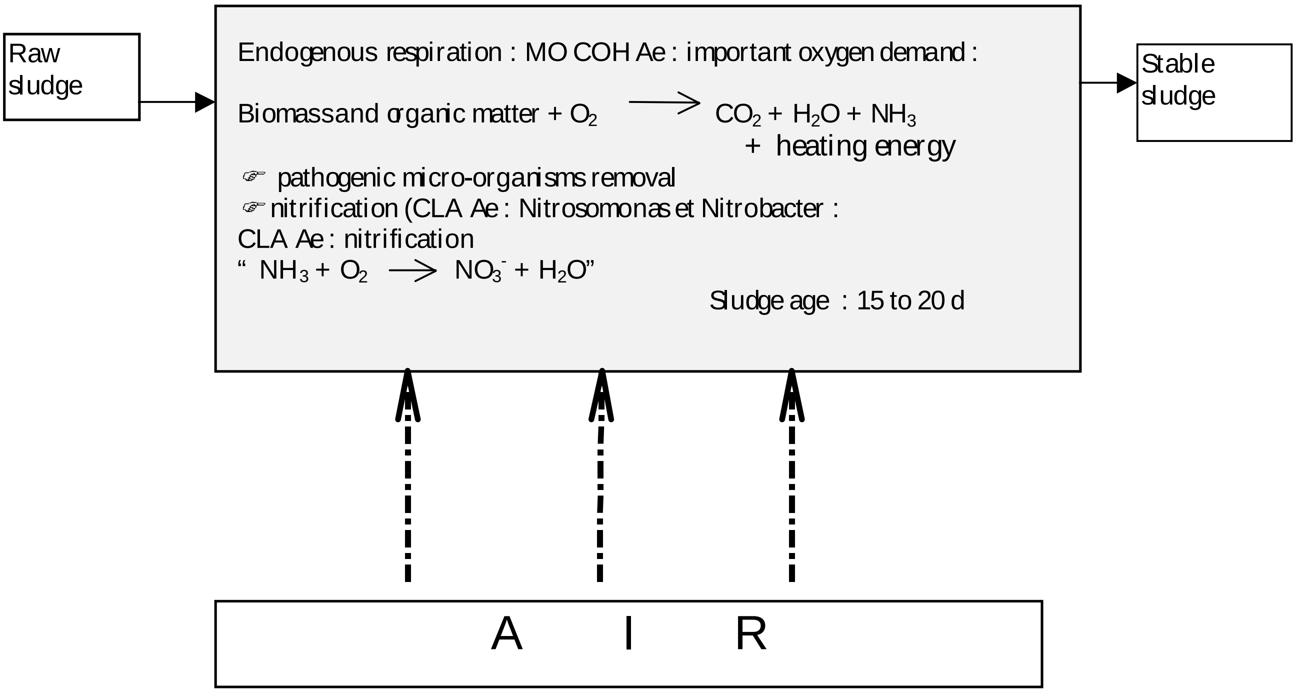


Figure 34 : sketch of a structure of aerobic stabilization of sludge

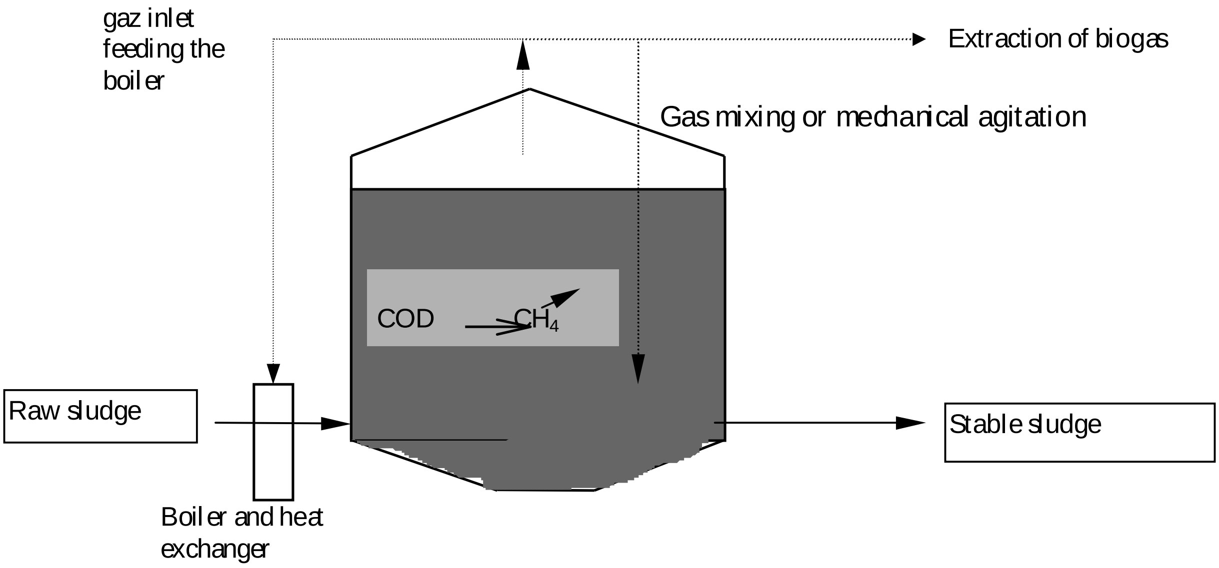


Figure 35 : Sketch of a moderate load digestor

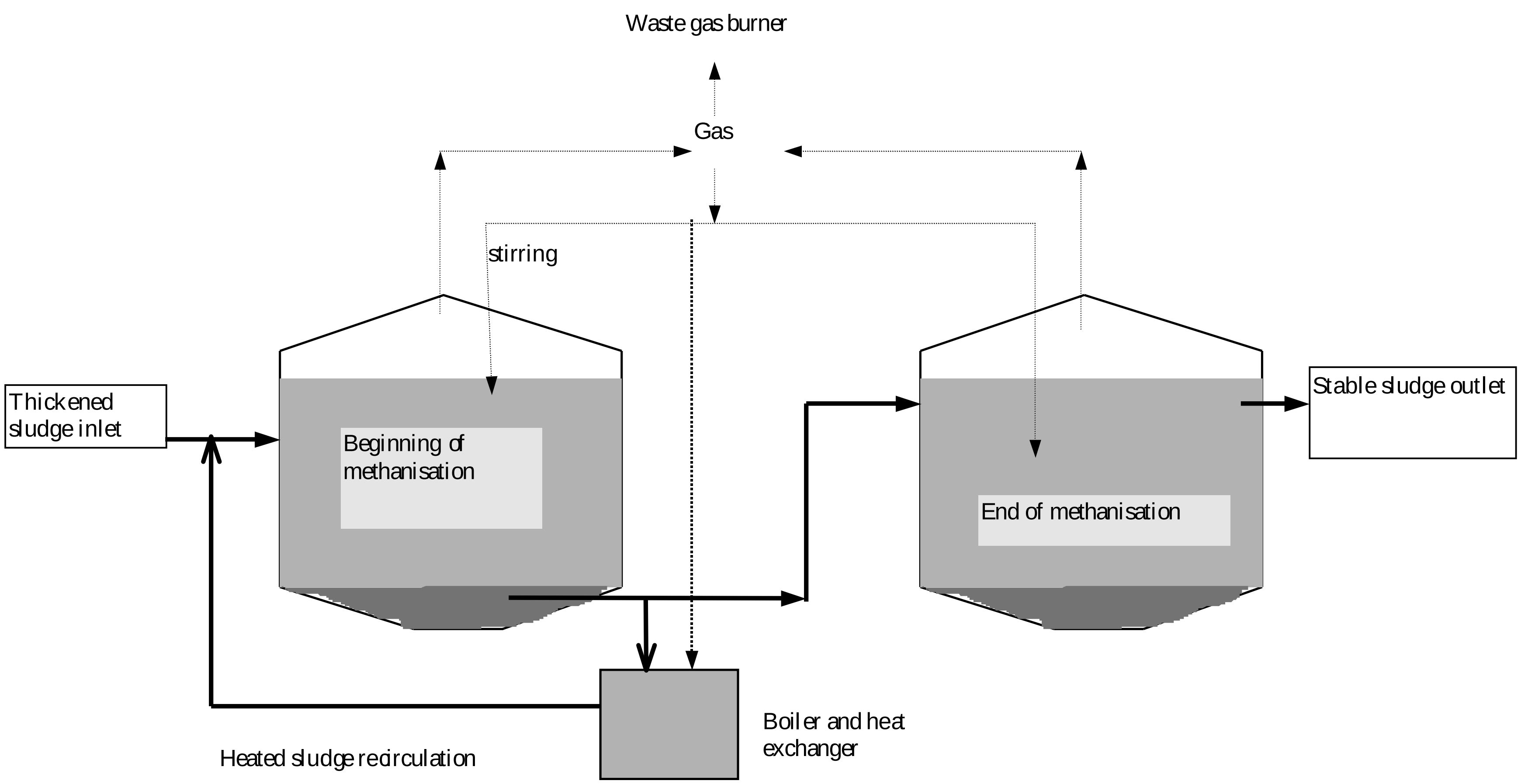


Figure 36 : Sketch of a high load digestor

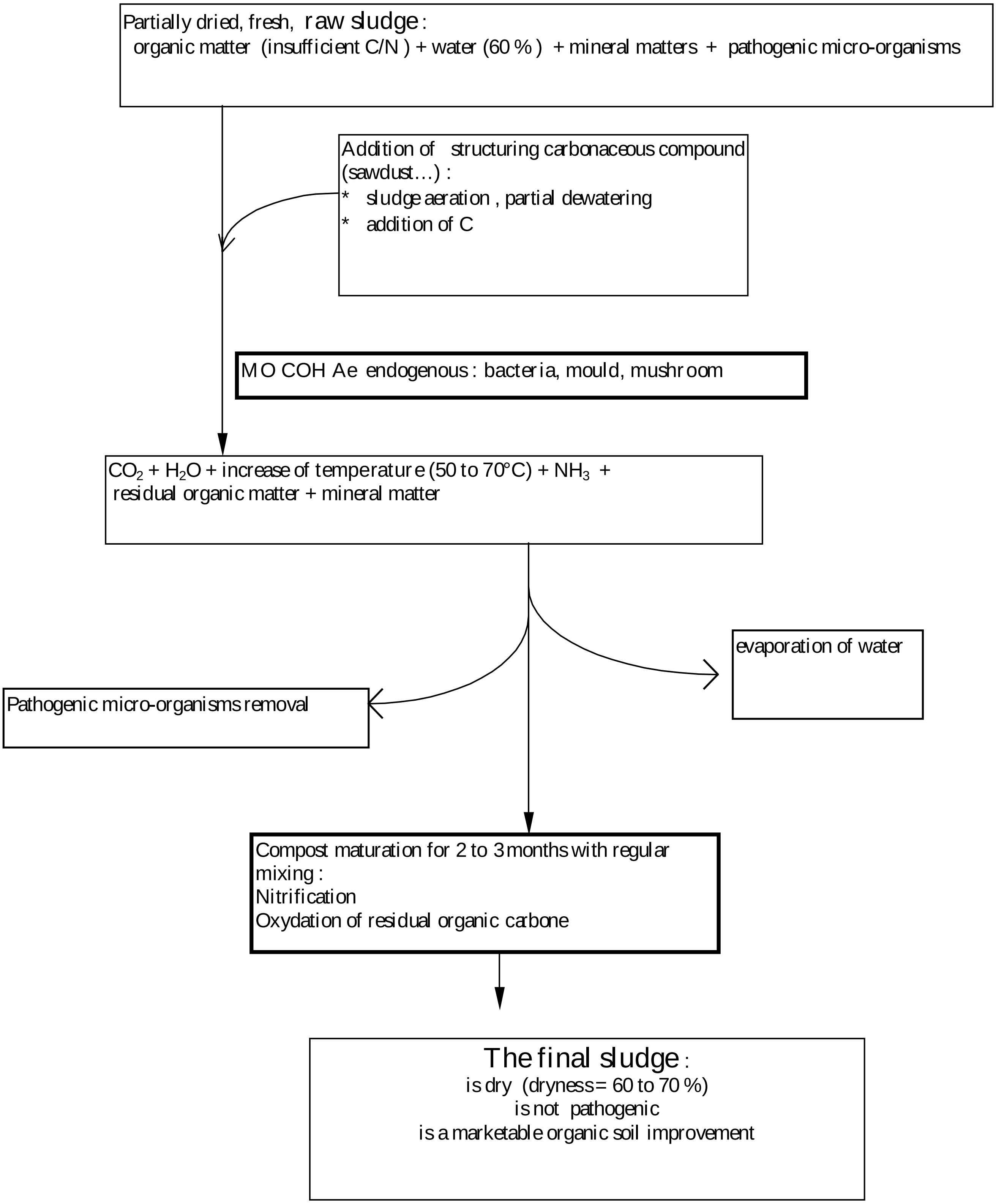


Figure 37 : Sketch of sludge composting

Undesirable compound C	Origin in MWW	Average concentration in MWW	Example of discharge standard	Toxicity or inconvenient	Physical removal	Biological process	Involved micro-organism	Nutri-tional type	Role of C in the metabolism Product of reaction (☞)	Condition for the treatment
Organic carbon : BOD_5	Organic matter	100 to 400 mgO_2/l	25 mgO_2/l	Consumption of O_2 in the medium	pre-treatment	* suspended cultures AS or AD (for agri food IWW) ** attached growth (D,Bf,Tf)	bacteria, proto and metazoa * flocculating * in biofilm : food chain	COH A	Source of electrons and C ☞ CO_2	* oxygenation * low load
Ammonia organic nitrogen	ammonifica-tion of urea, proteins… proteins, urea…	KN = 30 to 100 mg/l	NK + nitrates = GLN = 10 mg/l	consumption of O_2 toxic for fauna	by clarification : removal of C, N and P linked to SS	* suspended cultures AS ** attached growth (D,Bf,Tf)	Nitrosomonas and Nitrobacter	CLA Ae	Source of electrons ☞ NO_3^-	* oxygenation * low load * high sludge age
Nitrates	Absent in MWW Present in water after nitrification of KN	0		eutrophication		* suspended cultures AS with upstream anoxic zone ** attached growth Bf	miscellaneous : enterobacte-ria, Pseudomonas…	COH An	Final acceptor of electrons ☞ N_2	* efficient nitrification * anoxia * presence of organic matter
Phosphorus	Organic matter and washing powders…	10 to 25 mg/l	1 mg/l		precipitation with $FeCl_3$	AS with anaerobiosis zone	Aeromonas Acinetobacter Moraxella	COH Ae	Intracellular accumulation of P ☞ in sludge	* neither O_2 nor NO_3^- * aerobic sludge treatment

Figure 38 : Biological treatment of MWW

Caption : AS (activated sludge) ; AD (anaerobic digestion) ; D (disc) ; Bf (biofilter) ; Tf (trickling filter)