NCMA Medium 1: Artificial Sea Water-Zero valent iron plates

Medium appropriate for cultivating marine iron-oxidizing bacteria (FeOB).

Artificial Sea Water (ASW) per liter distilled H_2O:

NaCl	27.5 g
$MgCl_2$	5.38 g
$MgSO_4.7H_2O$	6.78 g
KCl	0.72 g
NH_4Cl	1.00 g
$CaCl_2.2H_2O$	1.40 g
K_2HPO_4	0.05 g (add after other constituents have dissolved)

This can be kept as an ASW stock solution.

The following are added when the assembling the final medium:

$NaHCO_3$	10 mM (0.84 g/L)
Mineral Solution	0.1% (See Recipe: NCMA Medium 7: Mineral solution)
Wolfe's vitamin solution	0.1% (See Recipe: NCMA Medium 6: Wolfe's Vitamin solution)

Zero valent iron (iron powder): (Alfa Aesar, -200 mesh cat # 00737-30)
Preparation:

Put about 10g of ZVI in each of 4 scintillation vials (or other cappable vials) and seal tightly. Place in an empty tip box with water in it up to the level of the powder in the vials.
Autoclave at 121C for 20 min, allow to cool; repeat total of 3x.
After autoclaving 3x, loosen the caps of the vials and put them in a desiccation chamber in case any steam made it into the vials. I leave them in the chamber until they are dry and then recap tightly, until use.

Preparation.
The $NaHCO_3$ and mineral solution can be added to the ASW prior to autoclaving. Autoclave the ASW medium, following autoclaving a precipitate will form due to presence of PO_4 & Mg salts at pH >7. Let this cool to room temperature (OK to put in cold water bath) and then adjust the pH to 6.5 by bubbling with a sterile stream of CO_2. The gassing time is dependent on the volume of medium and the flow rate of the CO_2 and can be determined empirically. Add the sterile vitamin mixture

Sprinkle the ZVI into each plate, approx. 0.1g/plate, and then add 15ml of medium. Inoculate either the bottle of ASW medium or each individual plate with cells. The plates are incubated in a sealed anaerobic jar (www.bd.com; GasPak 150 jars) with a GasPak EZ Campy Container System microaerophilic pouch (www. bd.com; cat # 260680). This system produces an atmosphere of approximately 5–10% O_2 and 5 to 12% CO_2 in the headspace. The entire top layer is inoculated with 2 to 3 ml of the desired organism per 100 ml media. Growth is indicated by accumulated Fe floc.. It is always necessary, however, to confirm the presence of FeOB by epifluoresence microscopy.