996. PERSEPHONELLA MEDIUM

NaCl	29.00	g
NaOH	2.00	g
KCl	0.50	g
$MgCl_2$ x 6 H_2O	1.36	g
$MgSO_4$ x 7 H_2O	7.00	g
$Na_2S_2O_3$ x 5 H_2O	2.00	g
$CaCl_2$ x 2 H_2O	0.40	g
NH_4Cl	0.20	g
K_2HPO_4 x 3 H_2O	0.30	g
Trace element stock solution (see below)	10.00	ml
Distilled water	990.00	ml

Trace element solution:

Na-EDTA x 2 H_2O	500	mg
$CoCl_2$ x 6 H_2O	150	mg
$MnCl_2$ x 4 H_2O	100	mg
$FeSO_4$ x 7 H_2O	100	mg
$ZnCl_2$	100	mg
$AlCl_3$ x 6 H_2O	40	mg
Na_2O_4W x 2 H_2O	30	mg
CuCl	20	mg
Ni_2SO_4 x 6 H_20	20	mg
H_2SeO_3 (selenous acid)	10	mg
H_3BO_3	10	mg
Na_2MoO_4 x 2 H_2O	10	mg

Adjust pH of trace elements to 3.

Medium is prepared with anoxic water (under a CO_2-atmosphere) and the pH is adjusted to 6 with H_2SO_4 prior to autoclaving. Medium is dispensed under a CO_2-atmosphere into Bellco tubes (5 ml medium per 27 ml tube) and stoppered with butyl stoppers, capped and crimped. After autoclaving a white precipitate might be present, this precipitate can be redissolved by shaking the medium. It can take up to an hour before all the precipitate is dissolved.
3.8% O_2 is added to each tube (1 ml of O_2 per 27 ml tube) and after inoculation the tubes were pressurized with H_2 to 20 psi (or 138 kPa). Inoculum should be in mid-logarithmic phase (16-10 h after inoculation). Incubation is carried out without shaking at 70°C and both strains (DSM 14351 and DSM 14350) grow within 24 h.